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THE IMMUNIZATION OF FOWLS AGAINST CHICKEN-POX (EPITHELIOMA CONTAGIOSUM) BY SUBCUTANEOUS INJECTION OF VIRUS

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INTRODUCTION[2]

The studies reported herein consist of a series of experiments in the immunization of fowls against chicken-pox (*Epithelioma contagiosum*) by the subcutaneous injection of vaccine containing the lesion tissue removed from fowls affected with the disease. The first report of a successful attempt to immunize fowls against chicken-pox by such means was that of Manteufel[(1)] in 1910. He reported success in immunizing fowls by injecting into the circulation or under the skin a lymph prepared from scraping of lesions on the comb or mucous membranes of the head of diseased birds mixed with physiologic salt solution and heated in a water bath at 55° C for one hour. According to this investigator, chickens treated in this manner were immune to infection for from one and one-half to two years even though there was no visible reaction following vaccination. Marked curative value for this preparation was also claimed.

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[2] Numerous papers reporting the results of experimental studies of chicken-pox and its control by vaccination have appeared in the literature. In this paper, however, reference is made only to studies pertaining to immunization of fowls against chicken-pox by the subcutaneous injection of a vaccine prepared from the lesions removed from diseased birds. For information regarding studies of the nature of the disease and its control by other methods of vaccination, readers are referred to the very excellent treatise by J. Verge, Recherches expérimenalés sur l'affection diphtéro–variolique des oiseaux. 230 p. J. Bonnet, Toulouse, France, 1926.

Hadley and Beach[2] in 1913 and Mack and Records[3, 4] in 1915 and 1916 reported the results of experiments with vaccine prepared after the method of Manteufel. They concluded that it was highly effective in the preventing of chicken-pox and exerted a curative effect on diseased fowls. No standard for the preparation of vaccine was adopted by any of the previously mentioned investigators.

The writer[5] in 1920 reported the results of the experimental use of vaccine prepared according to a modification of the Manteufel method. In this vaccine only the scabs from the chicken-pox lesions on the comb were used. This material was secured by artificial propagation on healthy cockrels. After removal, the scabs were dried and reduced to a fine powder. A standardized method of preparation was adopted. This consisted of mixing one gram of the dessicated virus with 100 cubic centimeters of sterile physiologic saline. The mixture was heated in a water bath at 55° C for one hour and preserved by the addition of 0.2 per cent of tricresol. This vaccine was reported to confer either immunity or increased resistance against infection with chicken-pox virus. Curative properties were also claimed for it. The immunity or resistance to infection after vaccination was not determined to be lasting, and, therefore, the vaccine was recommended for use in the control of outbreaks of chicken-pox in infected flocks rather than as a means of protecting healthy flocks against subsequent infection.

Experiments in the control of outbreaks of chicken-pox by Boerner and Stubbs,[6] reported in 1921, failed to confirm these results. These investigators concluded that vaccine prepared according to either method was not demonstrated to be of any value.

Quite different conclusions regarding the value of dry-virus vaccine, however, were reported by Fuller[7] in 1924 and Gwatkin[8] in 1925. Both of these investigators stated that their experiments demonstrated that the vaccine was of considerable value in controlling outbreaks of chicken-pox.

Vaccine prepared from dried virus has been very extensively used on poultry flocks in California in recent years. In the majority of cases satisfactory results have been obtained but not infrequently no apparent benefit was derived from vaccination. Such variation in results of the practical application of this method of vaccination on a large scale is in agreement with the results of the previously described experimental vaccination. The need for an improved method of preparation of vaccine is, therefore, apparent. It was for this purpose that the experiments described in the following pages were undertaken.

METHODS EMPLOYED

Certain of the methods of procedure were the same in all experiments. In order to avoid a repetition in the discussion of each experiment the following description of these methods is given at this point:

Material for the Preparation of Vaccine.—Material was secured from cockerels that had been inoculated with chicken-pox virus. The method of inoculation consisted in thoroughly scarifying the skin of both sides of the comb and applying a suspension of highly virulent chicken-pox virus to the scarified surface. Cockerels with the scarified area evenly covered with pronounced lesions were selected for use.

Virulence Tests.—Tests were made of all vaccines for the purpose of determining the presence of living virulent chicken-pox virus in the vaccines. They consisted of scarifying about 1 sq. cm. of the comb surface or of making four or five deep scratches in the skin of the comb with a large, dull hypodermic needle or a small trephine that had been dipped in the vaccine.

Immunizing Tests.—These tests consisted in vaccinating cockerels and later inoculating them with virulent chicken-pox virus. The method of vaccination was subcutaneous injection in the breast under the right wing with a 16 to 18-gauge hypodermic needle. The inoculation of the vaccinated birds was performed in the same manner as the virulence test except that the needle or trephine was dipped in a suspension of virulent virus instead of vaccine. Non-vaccinated, control cockerels, inoculated in the same manner with the same virus as the vaccinated birds, were included in each immunizing test. In all cases the virus used was found to be highly virulent.

In order that all of the experimental birds could with reasonable certainty be regarded as susceptible to chicken-pox, White Leghorn cockerels from ten to fourteen weeks old were used exclusively.

EXPERIMENTS WITH VACCINE PREPARED FROM WHOLE COMBS

This type of vaccine was prepared from the combs of cockerels that had been killed from nine to twelve days after inoculation with chicken-pox virus. Cockerels with nearly the entire comb surface evenly covered with chicken-pox lesions were selected. Three lots of

vaccine were prepared from such material. There were certain differences in the methods of preparing the vaccines as indicated in the following descriptions:

Vaccine No. 1.—Vaccine No. 1 was prepared on August 6, 1924, from the combs removed from two cockerels on the ninth day after inoculation with chicken-pox virus. The combs were cut into small pieces and triturated in a mortar with sterile sand and a small amount of 0.5-per-cent phenolized physiologic salt solution for more than three hours. Sufficient phenolized salt solution to provide 10 cc. for each gram of tissue was then added and the mixture filtered through gauze and filter paper. The resulting filtrate was a yellowish turbid liquid.

Vaccine No. 2.—Vaccine No. 2 was prepared on September 22, 1924, in the same manner as vaccine No. 1, from the combs removed from cockerels on the tenth day after inoculation with chicken-pox virus.

Vaccine No. 3.—Vaccine No. 3 was prepared March 24, 1925, from combs removed from cockerels on the tenth day after inoculation with chicken-pox virus. The diluent of this and later vaccines was a mixture of equal parts of glycerine and 1.0-per-cent phenolized physiologic salt solution. In a series of tests it was found that chicken-pox virus may remain alive in such a mixture for more than two years. It was thought therefore that it might be more desirable for use in the preparation of vaccine than the phenolized physiologic saline. The tissue was first put through a small Enterprise mill[3] (fig. 1) and then, with the addition of a small amount of diluent, through a special tissue mill (fig. 3) in which it was reduced to an extremely fine state. The mixture was next strained through a fine wire screen to remove the shreds of fibrous tissue that were not ground fine in the tissue mill. Sufficient of the glycerine-phenolized-saline mixture was then added to provide 10 cc. for each gram of tissue recovered from the Enterprise mill.

The results of the immunizing and virulence tests of the three vaccines appear in table 1.

[3] The Enterprise mill was later replaced by a Latapie grinder (fig. 2) which more satisfactorily prepared the tissue for the special tissue mill.

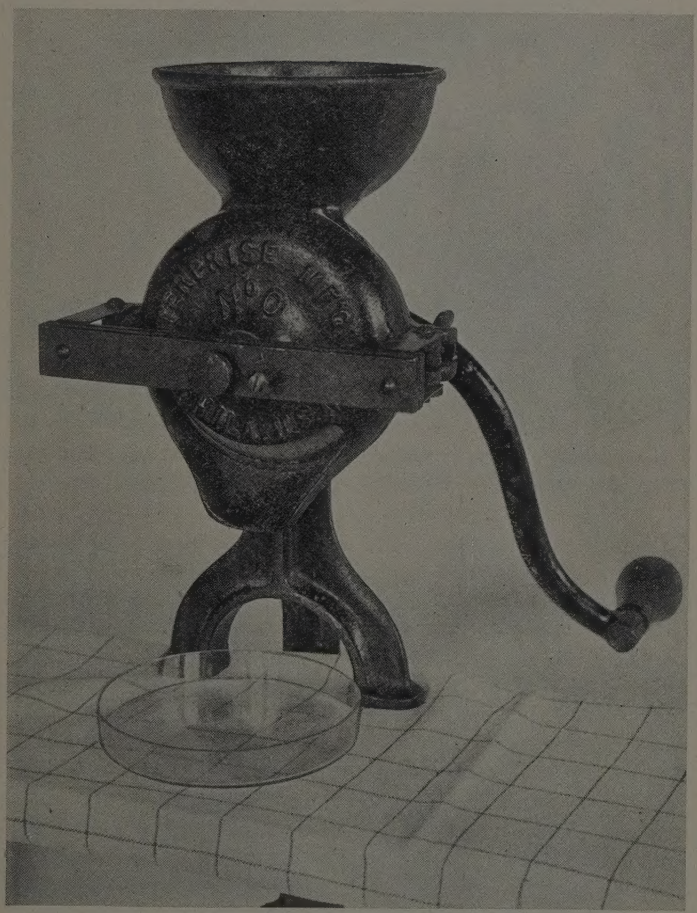

Fig. 1. Enterprise mill, No. 0, remodelled to permit removal of all grinding parts for cleaning and sterilizing. This mill is designed for grinding dry material and is not entirely satisfactory for fresh tissue.

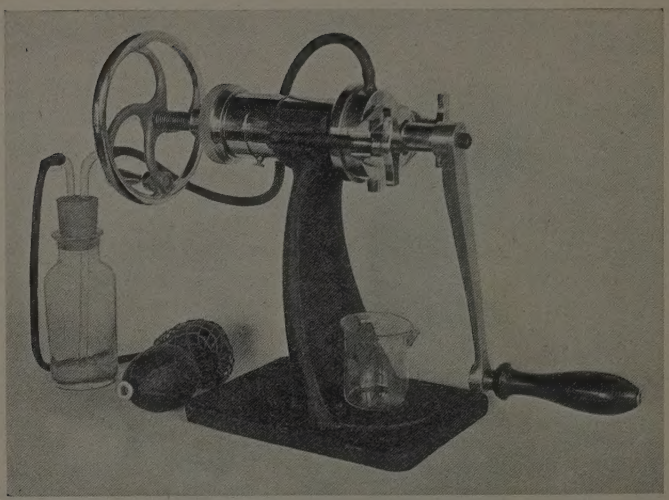

Fig. 2. Latapie grinder, designed especially for grinding fresh tissue. Procured from Cogit et Cie., Boulevard Saint-Michel, 36, Paris, France.

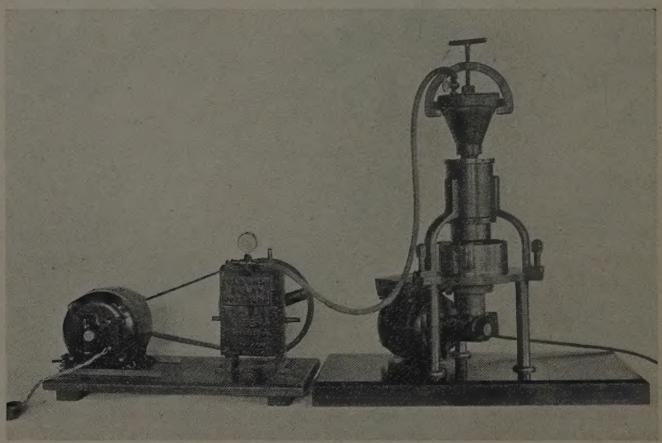

Fig. 3. A substantially constructed mill for fresh tissue, modelled after one used by the Cutter Laboratories, Berkeley, California, in the preparation of smallpox vaccine. Air pressure is used to hasten the passage of material through this mill.

TABLE 1

RESULTS OF IMMUNIZING AND VIRULENCE TESTS OF WHOLE-COMB VACCINES 1, 2, AND 3

Immunizing tests											Virulence tests			
Vaccine No.	Test No.	Vaccine: Age *days*	Vaccine: Number of doses	Vaccine: Am't of each dose *cc*	Number vaccinated	Number that developed chicken-pox lesions at vaccination point	Number that developed chicken-pox lesions on head	Number tested for immunity and days after vaccination when tested	Number immune or that developed chicken-pox	Number of controls inoculated	Lesions produced	Test No.	Age of vaccine *days*	Number inoculated	Lesions produced
1	1	1	1	1	4	....	0	2 in 14 days 2 in 28 days	1 slight lesions 1 marked lesions Both immune	2[a] 2[b]	Marked lesions on both. Moderate lesions on one Marked lesions on one	No virulence tests were made.			of vaccine No. 1
1	1	1	1	5	4	....	0	2 in 14 days 1[c] in 28 days	Both immune Both immune	2[a] 2[b]	Marked lesions on both Moderate lesions on one Marked lesions on one				
1	1	1	1	10	4	....	0	2 in 14 days 1[c] in 28 days	Both immune Bird immune	2[a] 2[b]	Marked lesions on both Moderate lesions on one Marked lesions on one				
1	2	25	2	2	10	....	0	10 in 14 days	7 immune 3 slight lesions	9	Marked lesions on all				
2	1	1	1	2	8	....	4	4 in 14 days 4 in 28 days	4 moderate lesions 4 immune	5[d] 4[e]	Marked lesions on all Marked lesions on all	No virulence tests were made at this age			of vaccine No. 2
2	1	1	2	2	8	....	3	2 in 14 days 4 in 28 days	2[f] slight lesions 4 immune	5[d] 4[e]	Marked lesions on all Marked lesions on all				

[a] Same controls for all groups inoculated on the same day.
[b] Same controls for all groups inoculated on the same day.
[c] One bird died from causes not related to vaccination or chicken-pox.
[d] The same 5 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 14 days after vaccination.
[e] The same 4 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 28 days after vaccination.
[f] Two birds died from causes not related to vaccination or chicken-pox.
[g] Three birds died from causes not related to vaccination or chicken-pox.
[h] These birds had not developed vaccine-injection-point lesions.

TABLE 1—(*Continued*)

Vaccine No.	Immunizing tests: Test No.	Vaccine: Age *days*	Vaccine: Number of doses	Vaccine: Am't of each dose *cc*	Number vaccinated	Number that developed chicken-pox lesions at vaccination point	Number that developed chicken-pox lesions on head	Number tested for immunity and days after vaccination when tested	Number immune or that developed chicken-pox	Number of controls inoculated	Lesions produced	Virulence tests: Test No.	Age of vaccine *days*	Number inoculated	Lesions produced
2	1	1	2	1	8	....	2	4 in 14 days	3 immune			No virulence test was made.			
									1 slight lesions	5[d]	Marked lesions on all				
								3[c] in 28 days	3 immune	4[e]	Marked lesions on all				
2	1	1	2	0.5	8	....	0	3[c] in 14 days	1 immune						
									2 slight lesions	5[d]	Marked lesions on all				
								2[g] in 28 days	2 immune	4[e]	Marked lesions on all				
2	2	29	2	0.5	20	....	0	20 in 14 days	20 slight lesions	19	Marked lesions on all	1	32	2	Marked lesions on both.
2	3	57	2	0.5	40	....	0	18[g] in 14 days	18 marked lesions 4 immune	20	Marked lesions on all	2	51	2	Marked lesions on both.
								17[h] in 28 days	13 slight to marked lesions	18	Marked lesions on all				
3	1	1	2	0.5	5	....	0	4[c] in 28 days	4 very slight lesions						
		1	2	1	5	....	0	4[c] in 28 days	4 immune	5	Marked lesions on all				
		1	2	2	5	....	0	4[c] in 28 days	4 immune						
3	2	....	1	0.5	3	3	0	2[c] in 28 days	2 immune						
		....	2	0.5	3	3	0	3 in 28 days	3 immune						
		25	1	1	3	3	0	3 in 28 days	3 immune	6	Marked lesions on all	1	25	2	Marked lesions on both.
		....	2	1	3	3	0	3 in 28 days	3 immune						
		....	1	2	3	3	0	3 in 28 days	3 immune						
		....	2	2	3	3	0	3 in 28 days	3 immune						

[a] Same controls for all groups inoculated on the same day.
[b] Same controls for all groups inoculated on the same day.
[c] One bird died from causes not related to vaccination or chicken-pox.
[d] The same 5 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 14 days after vaccination.
[e] The same 4 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 28 days after vaccination.
[f] Two birds died from causes not related to vaccination or chicken-pox.
[g] Three birds died from causes not related to vaccination or chicken-pox.
[h] These birds had not developed vaccine-injection-point lesions.

TABLE 1—(*Continued*)

Vaccine No.	Test No.	Immunizing tests: Vaccine: Age *days*	Vaccine: Number of doses	Vaccine: Am't of each dose *cc*	Number vaccinated	Number that developed chicken-pox lesions at vaccination point	Number that developed chicken-pox lesions on head	Number tested for immunity and days after vaccination when tested	Number immune or that developed chicken-pox	Number of controls inoculated	Lesions produced	Virulence tests: Test No.	Age of vaccine *days*	Number inoculated	Lesions produced
		....	1	0.5	3	3	0	3 in 28 days	3 immune						
		....	2	0.5	3	3	0	3 in 28 days	3 immune						
3	3	45	1	1	3	3	0	3 in 28 days	3 immune	6	Marked lesions on all	2	45	2	Marked lesions on both.
		....	2	1	3	3	0	[c] in 28 days	2 immune						
		....	1	2	3	3	0	3 in 28 days	3 immune						
		....	2	2	3	3	0	3 in 28 days	3 immune						
		....	1	0.1	3	[c]	0	[c] in 28 days	2 immune						
		....	2	0.1	3	0	0	3 in 28 days	3 immune						
		....	1	0.5	3	2	0	3 in 28 days	3 immune						
3	4	86	2	0.5	3	2	0	3 in 28 days	3 immune	6	Marked lesions on all	3	86	2	Marked lesions on both.
		....	1	1	3	3	0	3 in 28 days	3 immune						
		....	2	1	3	0	0	3 in 28 days	1 immune [h] moderate lesions						
		86	1	2	3	3	0	3 in 28 days	3 immune						
		86	2	2	3	2	0	3 in 28 days	2 immune [h] moderate lesions						
		.....	1	0.5	3	0	0	3 in 28 days	All marked lesions						
		.....	2	0.5	3	0	0	3 in 28 days	All marked lesions						
3	5	183	1	1	3	0	0	3 in 28 days	All marked lesions	6	Marked lesions on all	4	183	2	None.
		.....	2	1	3	0	0	3 in 28 days	All marked lesions						
		.....	1	2	3	0	0	3 in 28 days	All marked lesions						
		.....	2	2	3	0	0	3 in 28 days	All marked lesions						

[a] Same controls for all groups inoculated on the same day.
[b] Same controls for all groups inoculated on the same day.
[c] One bird died from causes not related to vaccination or chicken-pox.
[d] The same 5 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 14 days after vaccination.
[e] The same 4 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 28 days after vaccination.
[f] Two birds died from causes not related to vaccination or chicken-pox.
[g] Three birds died from causes not related to vaccination or chicken-pox.
[h] These birds had not developed vaccine-injection-point lesions.

Discussion of Results.—Eight of the twelve cockerels that received a subcutaneous injection of 1 cc., 5 cc., or 10 cc. of fresh vaccine No. 1 were immune to artificial infection with chicken-pox virus 14 or 28 days later. Two of the cockerels that were inoculated 14 days after vaccination with a 1-cc. dose of vaccine developed chicken-pox lesions which, however, were less marked than those which developed on the controls. Two of the cockerels were lost to the experiment from causes not related to vaccination. Seven of ten cockerels vaccinated with two 2-cc. doses of the vaccine, 25 days after preparation were found to be immune to chicken-pox when they were inoculated 14 days after vaccination. Slight chicken-pox lesions developed in the remaining three cockerels. No harmful effect or reaction of any nature was observed in any of the birds after vaccination.

Nine of the twenty-four birds that were vaccinated with one or two 2-cc. doses or two 1-cc. doses of the fresh vaccine No. 2 developed one or two small chicken-pox tumors on the comb or wattles in from 12 to 18 days after vaccination. These lesions did not spread and disappeared in about 10 days. Ten of these twenty-four birds were inoculated 14 days after vaccination. Three of the ten were immune. Seven developed slight or moderate chicken-pox lesions. Eleven of the twenty-four birds were inoculated in 28 days after vaccination. All were immune. Three birds died before the time for inoculation arrived. None of the eight birds that received two 0.5-cc. doses of fresh vaccine became infected from the vaccine. Inoculation with chicken-pox virus on the fourteenth day after vaccination with three birds and the twenty-eighth day after vaccination with two birds, however, showed them to be just as resistant to the infection as the birds that had received larger doses of vaccine. This made it appear that such a dosage might be more satisfactory than a larger one. Consequently twenty cockerels were given two 0.5-cc. doses with 20-day-old vaccine. They were inoculated 14 days after vaccination. All were highly resistant to the infection but none were entirely immune. Another lot of forty birds were vaccinated with the same dose when the vaccine was 57 days old. None became infected from the vaccine. Five were lost from the experiment by death from causes unrelated to vaccination. Eighteen were inoculated on the fourteenth day after vaccination. All of these developed marked chicken-pox lesions. Seventeen were inoculated on the twenty-eighth day after vaccination. Four were immune. The others developed slight to marked lesions. These results indicate that the vaccine was decreasing in immunological value as it increased in age. It is presumed that the immunological value is to a large degree dependent upon the amount and

virulence of the virus present. Tests failed to demonstrate that the virulence in the 57-day-old vaccine was less than in that 32 days old. Experience has shown, however, that fowl inoculation would detect only marked differences in virulence.

In the experiments with vaccine No. 3, all inoculations of vaccinated birds with chicken-pox virus were made on the twenty-eighth day after vaccination, since the results with vaccine No. 2 had indicated that this was a more suitable time than the fourteenth day.

Comparative tests of one and two doses of 0.1 cc., 0.5 cc., 1 cc., and 2 cc. of vaccine and of 1-day, 25, 45, 86, and 183-day old vaccine were performed on a total of ninety-three birds.

No chicken-pox lesions appeared about the head of any bird as a result of the injection of vaccine. In the course of the examinations of the first lot of birds vaccinated, however, a dry scab resembling a chicken-pox lesion was observed on the skin of some birds at the point of vaccination. The scab was removed, ground in a mortar with sterile saline and applied to a scarified surface of the skin of two normal cockerels. Marked chicken-pox lesions were produced, thus definitely establishing that the lesions observed were chicken-pox. This observation was made too late to permit the securing of data on the occurrence of such lesions on this lot of birds. Such data were secured, however, from all birds that were vaccinated later.

Vaccine-injection-point chicken-pox lesions appeared on all but nine of the sixty cockerels that were vaccinated with the 25, 45, or 86-day-old vaccine. The exceptions were all among those that received the 86-day-old vaccine. Three of the nine received two 0.1-cc. doses, one received one 0.5-cc. dose, one received two 0.5-cc. doses, three received two 1-cc. doses, and one received one 2-cc. dose. It is seen, therefore, that no relationship existed between the size of the dose of vaccine and the development of vaccine-injection-point lesions. In those birds that received two doses of vaccine the lesions appeared only at the point of the first injection. They reached sufficient size to be definitely recognized in from 7 to 10 days and persisted for from 10 to 20 days. In all cases the lesions had healed when the fowls were inoculated with chicken-pox virus on the twenty-eighth day after vaccination. In most instances the vaccine-injection-point lesion consisted of a single tumor from 2 mm. to 4 mm. in diameter. On a few birds, however, the lesions covered an area as large as from 10 mm. to 15 mm. in diameter. In no case did the infection become general or in any way harm the fowls.

Six birds were lost from the experiment by death. The immunity test was completed with eighty-seven birds.

Of sixty-nine birds in the test of 1, 25, 45, or 86-day-old vaccine, sixty-two were immune and seven developed slight chicken-pox lesions after inoculation on the twenty-eighth day after vaccination. Of the seven birds that were slightly susceptible to the infection, four had received two 0.5-cc. doses of 1-day-old vaccine and two had received two 1-cc. doses and one had received two 2-cc. doses of 86-day-old vaccine. The failure of these birds to become entirely immunized cannot be ascribed either to the size or number of the doses or to the age of the vaccine, since other birds were immunized by a smaller dose of vaccine of the same age or older.

Three of the non-immune birds were from those that did not develop vaccine-injection-point lesions. This cannot be considered the reason for their remaining slightly susceptible to chicken-pox, however, because six other birds that had had no vaccination lesions were found to be immune.

The data concerning the sixty-eight birds that were immunized by vaccination show that doses of 0.1 cc., 0.5 cc., 1 cc., and 2 cc. were equally effective, that one dose of any of these amounts was as effective as two doses, and that no apparent decrease in the effectiveness of the vaccine had occurred by keeping it for 86 days. The virulence tests showed no discernable difference in the virulence of the virus contained in the 25, 45, and 86-day-old vaccine. The 183-day-old vaccine failed to produce any vaccination lesions, to immunize or increase the resistance to chicken-pox of any of the eighteen birds that were vaccinated with it, or to produce any lesions on the fowls used in the virulence test.

Vaccine No. 3 after 86 days storage proved to be a more potent immunizing agent than vaccine No. 2 after 57 days storage. The only essential difference between the two vaccines was that the glycerine-phenolized-saline mixture was used in the preparation of the former and phenolized saline alone in the latter. This suggests that the mixture of equal parts of glycerine and 1.0-per-cent phenolized salt solution is more suitable for use than the phenolized saline alone.

The results of this series of experiments show that fowls can be immunized against artificial infection with chicken-pox virus in 28 days by subcutaneous injection with vaccine prepared from fresh comb and lesion tissue of cockerels with marked chicken-pox infection.

Evidence was also procured to show that, although the vaccine contains virulent chicken-pox virus and its injection is liable to be attended by the development of slight chicken-pox lesions on the skin at the point of injection, it is not liable to cause infection at other locations, such as about the head.

EXPERIMENTS WITH VACCINES PREPARED FROM WHOLE COMBS, BLOOD, AND ORGANS

The purpose of the experiments with vaccines Nos. 4, 5, and 6 was to determine if the inclusion of the blood, liver, spleen, and kidneys of cockerels with marked chicken-pox infection with the comb and lesion tissue would result in a vaccine of greater immunizing value than one containing comb and lesion tissue only.

Vaccine No. 4.—The first lot of this type of vaccine, No. 4, was prepared on March 24, 1925. A cockerel was killed in a gas chamber on the tenth day after inoculation with chicken-pox virus. The abdominal and thoracic cavities were immediatley opened, the aorta severed and the blood allowed to collect in the abdominal cavity. The blood, liver, spleen, kidneys, and comb were removed for the preparation of the vaccine. The method of preparation was the same as that of vaccine No. 3. The proportion of diluent to tissue was 10 cc. per gram. The weight of the blood and organs was 20 grams and of the comb and lesion tissue 16 grams. Each cubic centimeter of this vaccine, therefore, contained less than half the amount of the comb and lesion tissue in a like amount of vaccine No. 3.

Immunizing and virulence tests were made with fresh vaccine and vaccine 25, 45, 86, and 183 days old. In all cases the cockerels used in the immunizing tests were inoculated with chicken-pox virus on the twenty-eighth day after vaccination.

The results of the immunizing and virulence tests are summarized in table 2.

As shown in table 2, all of the sixty-nine cockerels that were given one or two subcutaneous injections of 0.1 cc., 0.5 cc., 1 cc., or 2 cc., each of vaccine No. 4, when it was 1, 25, 45, or 86 days old, were immune to artificial infection with chicken-pox virus 28 days later. The eighteen birds that were vaccinated with one or two 0.5-cc., 1-cc., or 2-cc. doses of 183-day-old vaccine exhibited neither immunity nor resistance to artificial infection 28 days later. The same results were obtained with the smallest dose and the largest dose of vaccine, and with one dose and two doses.

The results of the virulence tests showed that the 1, 25, 45, and 86-day-old vaccine contained virulent chicken-pox virus and did not indicate that the virulence had decreased with age. No virulence was demonstrated in the 183-day-old vaccine.

TABLE 2

RESULTS OF IMMUNIZING AND VIRULENCE TESTS OF VACCINE NO. 4

Test No.	Immunizing tests: Vaccine: Age *days*	Vaccine: Number of doses	Vaccine: Amount of each dose *cc*	Number vaccinated	Number that developed vaccination-point lesions[a]	Number tested for immunity 28 days after vaccination	Number immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Virulence tests: Test No.	Age of vaccine *days*	Number inoculated	Lesions produced
1	1	2	0.5	5	.....	3[b]	3 immune	5	Marked lesions on all	1	1	2	Marked
		2	1	5	.....	3[b]	3 immune						
		2	2	5	.....	5	5 immune						
2	25	1	0.5	3	3	3	3 immune	6	Marked lesions on all	2	25	2	Marked
		2	0.5	3	3	3	3 immune						
		1	1	3	3	2[c]	2 immune						
		2	1	3	3	3	3 immune						
		1	2	3	3	3	3 immune						
		2	2	3	3	3	3 immune						
3	45	1	0.5	3	3	3	3 immune	6	Marked lesions on all	3	45	2	Marked
		2	0.5	3	3	3	3 immune						
		1	1	3	3	3	3 immune						
		2	1	3	3	2[c]	2 immune						
		1	2	3	3	3	3 immune						
		2	2	3	3	3	3 immune						

[a] No examination for vaccination-point lesions were made at the first test.
[b] Two died from causes not related to vaccination.
[c] One died from causes not related to vaccination.

TABLE 2—(*Continued*)

Test No.	Immunizing tests: Vaccine: Age *days*	Vaccine: Number of doses	Vaccine: Amount of each dose *cc*	Number vaccinated	Number that developed vaccination-point lesions[a]	Number tested for immunity 28 days after vaccination	Number immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Virulence tests: Test No.	Age of vaccine *days*	Number inoculated	Lesions produced
4	86	1	0.1	3	2	3	3 immune	6	Marked lesions on all	4	86	2	Marked
		2	0.1	3	1	3	3 immune						
		1	0.5	3	3	3	3 immune						
		2	0.5	3	0	3	3 immune						
		1	1	3	1	3	3 immune						
		2	1	3	1	3	3 immune						
		1	2	3	0	3	3 immune						
		2	2	3	0	3	3 immune						
5	183	1	0.5	3	0	3	3 marked lesions	6	Marked lesions on all	5	183	2	None
		2	0.5	3	0	3	3 marked lesions						
		1	1	3	0	3	3 marked lesions						
		2	1	3	0	3	3 marked lesions						
		1	2	3	0	3	3 marked lesions						
		2	2	3	0	3	3 marked lesions						

[a] No examination for vaccination-point lesions were made at the first test.
[b] Two died from causes not related to vaccination.
[c] One died from causes not related to vaccination.

Chicken-pox lesions at the point of injection of vaccine developed on all of the thirty-six cockerels that were vaccinated with the 25 and 45-day-old vaccines and on nine of the twenty-four birds that received the 86-day-old vaccine. These lesions could be definitely identified from 7 to 12 days after vaccination. They were entirely healed by from 20 to 28 days after vaccination. No lesions developed elsewhere on any of the birds. All of the seventeen birds vaccinated with the 86-day-old vaccine that did not develop lesions at the point where the vaccine was injected were immune to infection with chicken-pox virus 28 days after vaccination. This is additional evidence that the development of vaccine-injection-point lesions is not essential to the production of immunity. There were no vaccination-point lesions on the birds vaccinated with 183-day-old vaccine.

Vaccine No. 4 was prepared on the same date and tested in the same manner and on the same dates as vaccine No. 3. The immunizing tests of vaccine No. 4 yielded slightly better results than the corresponding tests with vaccine No. 3, although the former contained less than half as much comb and lesion tissue as the latter. The difference was not sufficiently marked, however, to warrant the conclusion that a vaccine prepared from the blood, liver, spleen, kidneys, comb, and lesions of a cockerel with marked chicken-pox infection of the comb is superior to one prepared from the comb and lesion tissue only as an agent for immunizing to chicken-pox infection. Virulence tests of the two vaccines gave identical results.

Vaccines 5 and 6.—For further comparison of the immunizing value of a vaccine containing the blood, organs, and comb and lesion tissue with one containing comb and lesion tissue only, two vaccines, Nos. 5 and 6, were prepared. The methods of preparation were the same as used for vaccines Nos. 3 and 4. Vaccine No. 5 contained the comb and lesion tissue and vaccine No. 6 the comb and lesions, blood, liver, spleen, and kidneys of cockerels that were killed on the ninth day after inoculation with chicken-pox virus. In each vaccine the proportion of the diluent to the tissue was 10 cc. to 1 gram. Each cubic centimeter of vaccine No. 5, therefore, contained more of the comb and lesion tissue than vaccine No. 6. In the immunizing and virulence tests this difference in the two vaccines was overcome by diluting them sufficiently with the glycerine-phenolized-saline mixture to make 1 cc. of each contain the same amount of comb and lesion tissue.

The results of the tests of vaccines Nos. 5 and 6 are given in table 3.

TABLE 3

RESULTS OF IMMUNIZING AND VIRULENCE TESTS OF VACCINES 5 AND 6[a]

Immunizing tests								Virulence tests	
Vaccine			Number of fowls vaccinated	Number that developed vaccination-point lesions	Number tested for immunity 28 days after vaccination	Number immune	Number of controls inoculated	Lesions produced	Number inoculated with vaccine	Lesions produced
No.	Type of tissue	Amount of comb tissue in dose[b] *grams*								
5	Comb and lesion	0.063	3	3	2[c]	2	3	Marked	2	Marked
		.0093	3	2	3	3			2	Marked
		.00093	3	1	3	3			2	1 tumor on each bird.
6	Comb and lesions, blood, and organs	.0093	3	3	2[c]	2	3	Marked	2	Marked
		0.00093	3	3	3	3			2	None

[a] Vaccines were 4 days old when tests were made.
[b] Size of dose was uniformly 1 cc.
[c] One died from causes not related to vaccination.

As shown in table 3, all of the thirteen cockerels that were inoculated with chicken-pox virus in 28 days after vaccination were immune to the infection, irrespective of the type or amount of tissue in the vaccine.

Lesions developed at the point of injection of vaccine on six of the nine birds that received vaccine No. 5 and on all of six birds that received vaccine No. 6. The birds that did not develop vaccination lesions were one of three that received 0.0093 gram of comb tissue and two of three that received 0.00093 gram of comb tissue. Failure of vaccination lesions to appear, therefore, was not confined to those birds that received the smallest amount of comb and lesion tissue. These three birds were immune to infection with chicken-pox virus 28 days after vaccination, thus adding to the data showing that the development of lesions at the vaccination point is not essential to the development of immunity.

The virulence test demonstrated the presence of highly virulent virus in all dilutions of vaccine. The fact that but one tumor was produced on each of two birds and no lesions on two others that were inoculated with the dilutions containing 0.00093 gram of comb tissue per cubic centimeter, however, indicates that there was only a small amount of virus in that dilution.

The results of this experiment have failed to demonstrate any difference in the immunizing value of the two vaccines, both of which contained like amounts of comb and lesion tissue and one of which contained, in addition, the blood, liver, spleen, and kidneys of cockerels with marked chicken-pox infection.

EXPERIMENTS WITH VACCINES PREPARED FROM THE LESIONS OF THE COMB AND FROM THE BLOOD AND ORGANS

In the preceding comparative experiments it was found that vaccines prepared from the whole comb or from the whole comb, blood, liver, spleen, and kidneys of cockerels with marked chicken-pox lesions appeared to be equally effective in immunizing fowls against artificial infection with chicken-pox. It was not determined, however, whether the inclusion of the blood and organs added to the immunizing properties of vaccine. To obtain information on this point it therefore appeared necessary to prepare vaccines from such tissue alone and to make comparisons of the immunizing properties of such vaccines with others prepared from comb and lesion tissues.

For this purpose a series of five vaccines, Nos. 7, 8, 9, 10, and 11, were made from the blood, livers, spleens, and kidneys of the birds killed on the tenth, eleventh, twelfth, thirteenth, and fourteenth days, respectively, after inoculation and five other vaccines, Nos. 12, 13, 14, 15, and 16, from the lesions of the combs of the same birds. Birds were selected that had a uniform, heavy growth of chicken-pox lesions covering the surface of both sides of the comb, but that did not exhibit general symptoms of sickness, such as marked droopiness and inappetence.

The method of preparing the vaccines from the blood and organs was essentially the same as that of previous preparations. Sufficient diluent was added to make each cubic centimeter of vaccine contain 0.33 gram of tissue.

For the preparation of the vaccines from comb tissue, instead of the whole comb, the lesions and sublesion epithelial tissue only were used. This tissue was removed with a sharp scalpel, care being taken to include as little as possible of the subcutaneous fibrous tissue. The reasons for making this change were that the tough fibrous portions of the comb had proved very difficult to reduce to a fine state, that much of it had been removed when the vaccine was strained, and that the portion of it remaining in the vaccine was probably inert. The method of preparing the vaccines was otherwise the same as that previously described. The amount of the lesion tissue that was not ground finely enough to pass through the screen was negligible. Sufficient diluent was added so that 1 cc. of vaccine contained 0.1 gram of tissue.

In addition to determining the immunizing value of the vaccines these experiments were designed to furnish information regarding the presence of chicken-pox virus in the blood and internal organs of infected fowls; the minimum amount of lesion tissue which, when injected subcutaneously, will produce immunity to chicken-pox; and the most suitable time after inoculation to secure material for the preparation of vaccine from lesion tissue.

Some information on the latter question was furnished by the yield of lesion tissue by the birds killed on the different days after inoculation. The birds and the area of comb surface occupied by lesions were approximately the same size. The amount of tissue obtained was 9, 13, 19, 16, and 15 grams from the bird killed on the tenth, eleventh, twelfth, thirteenth, and fourteenth day, respectively, after inoculation. The increase in the amount of tissue from the tenth to the twelfth day was due to increase in the thickness of the lesion

TABLE 4

RESULTS OF FIRST IMMUNIZATION AND VIRULENCE TESTS OF VACCINES 7 TO 16. (Fresh vaccine used)

Vaccine			Immunizing tests							Virulence tests		
No.	Prepared from	Amount of tissue in dose *grams*	Number vaccinated	Number developed vaccination-point lesions	Number developed lesions on the head	Number tested for immunity 28 days after vaccination	Number immune or developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Number inoculated	Lesions produced	Incubation period *days*
7	Blood and organs from cockerel	0.33	1	0	1[a]	1	1 immune[b]	2	Marked	1	Marked	10
	10 days after inoculation	.66	1	0	0	1	Lesions produced[c]					
8	Blood and organs from cockerel	.33	1	0	0	1	1 immune	2	Marked	1	A few tumors	9[d]
	11 days after inoculation	.66	1	0	0	1	Lesions produced					
9	Blood and organs from cockerel	.33	1	1	0	1	1 immune	2	Marked	1	Marked	8
	12 days after inoculation	.66	1	0	0	1	1 immune					
10	Blood and organs from cockerel	.33	1	0	0	1	1 immune	2	Marked	1	Marked	8
	13 days after inoculation	.66	1	1[e]	0	1	1 immune					
11	Blood and organs from cockerel	.33	1	0	0	1	1 immune	2	Marked	1	Marked	15[d]
	14 days after inoculation	.66	1	0	0	1	1 immune					

[a] Lesions probably not produced by the vaccine.

[b] Immunity probably due to comb infection rather than to the vaccine.

[c] Bird died 10 days after inoculation. Lesions active at time of death.

[d] Lesions were present at the same time on the opposite side of the comb. All lesions may therefore be due to accidental infection. If the lesions on the opposite side of the comb had resulted from inoculation, they would not have appeared until after the lesion at the point of inoculation.

[e] Lesions did not develop until the 24th day after vaccination. May not have been caused by the vaccine.

[f] These lesions not observed until the 28th day after vaccination. May have resulted from accidental infection rather than vaccination.

TABLE 4—(*Continued*)

Immunizing tests										Virulence tests		
Vaccine												
No.	Prepared from	Amount of tissue in dose *grams*	Number vaccinated	Number developed vaccination-point lesions	Number developed lesions in the head	Number tested for immunity 28 days after vaccination	Number immune or developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Number inoculated	Lesions produced	Incubation period *days*
12	Lesions from cockerel 10 days after inoculation	.1	2	2	0	2	2 immune	2	Marked	1	Marked	6
		.03	2	2	1[f]	2	2 immune			1	Marked	6
13	Lesions from cockerel 11 days after inoculation	.1	2	2	0	2	2 immune	2	Marked	1	Marked	6
		.01	2	2	0	2	2 immune			1	Marked	6
14	Lesions from cockerel 12 days after inoculation	.1	2	2	0	2	2 immune	2	Marked	1	Marked	6
		.005	2	2	1[f]	2	2 immune			1	Marked	6
15	Lesions from cockerel 13 days after inoculation	.1	2	2	0	2	2 immune	2	Marked	1	Marked	6
		.002	2	2	0	2	2 immune			1	Marked	6
16	Lesions from cockerel 14 days after inoculation	.1	2	2	0	2	2 immune	2	Marked	1	Marked	6
		0.001	2	2	0	2	2 immune			1	Marked	6

[a] Lesions probably not produced by the vaccine.

[b] Immunity probably due to comb infection rather than to the vaccine.

[c] Bird died 10 days after inoculation. Lesions active at time of death.

[d] Lesions were present at the same time on the opposite side of the comb. All lesions may therefore be due to accidental infection. If the lesions on the opposite side of the comb had resulted from inoculation, they would not have appeared until after the lesion at the point of inoculation.

[e] Lesions did not develop until the 24th day after vaccination. May not have been caused by the vaccine.

[f] These lesions not observed until the 28th day after vaccination. May have resulted from accidental infection rather than vaccination.

tissue. The decrease in amount after this time appeared to be the result of the beginning of dry-scab formation. Assuming that the tissue from these birds is of equal value for vaccine preparation, the most suitable time for securing tissue is shown to be when the lesions have reached their maximum development and before drying has begun. In this case, it was on the twelfth day after inoculation.

First Immunizing and Virulence Tests of Vaccines 7 to 16.—For the determination of the immunizing properties of the vaccines, groups of from two to four fowls were given a subcutaneous injection of vaccine and inoculated with chicken-pox virus 28 days later. Since the results of previous experiments had indicated that one dose of vaccine was as effective as two doses given seven days apart, one dose only was used in this experiment.

In all tests of the blood-and-organ-tissue vaccine, the dose was 1 cc. or 2 cc. of the vaccine as prepared. Each cubic centimeter contained 0.33 grams of tissue. The dose of skin-and-lesion-tissue vaccine was 1 cc. containing 0.1, 0.03, 0.01, 0.005, 0.002, or 0.001 grams of tissue. The variation in amount of tissue was accomplished by diluting the original preparation with glycerine-phenolized saline mixture so that 1 cc. of the dilution contained the amount of tissue it was desired to administer. The dilutions were always made just before use.

In the virulence tests one cockerel was inoculated with the same concentrations of vaccine used in the immunizing tests.

The results of the first tests are given in table 4.

As indicated in table 4, chicken-pox lesions that appeared on some of the birds that may have resulted from infection with virus other than that contained in the vaccine. Evidence that such virus was present is furnished by the fact that chicken-pox occurred among normal cockerels that were in another compartment of the same house. The irregularity of the occurrence of chicken-pox lesions on some of the birds with respect to the time of vaccination or inoculation provided an additional reason for believing that infection of some of the birds was accidental. Accurate interpretation of the results of these experiments is, therefore, rendered difficult, and for this reason they will not be discussed in detail.

However, the results of this experiment have again demonstrated that vaccine prepared from fresh lesion tissue and containing virulent chicken-pox virus when injected subcutaneously in fowls is capable of producing immunity to artificial infection with chicken-pox virus. The injection is liable to be followed by the occurrence at the point of injection of chicken-pox lesions which are slight and do not constitute a harmful infection. The significance of such lesions in the development of immunity is still undetermined since, in preceding experiments, immunity was produced without the occurrence of them. The smallest amount of lesion tissue that will immunize remains unknown since 0.001 gram was as effective in this respect as 0.1 gram.

The results with vaccines prepared from blood and internal organ tissue suggest that they contained virulent chicken-pox virus and possessed immunizing properties. However, on account of the occurrence on some of the experimental birds of chicken-pox lesions that apparently resulted from accidental infection, definite conclusions could not be drawn.

Second Immunizing and Virulence Tests of Vaccines 7 to 16.—To avoid interference from accidental infection of the experimental fowls such as was encountered in the preceding experiment, these tests were carried out under more carefully controlled conditions.

The results of the tests are given in table 5.

The data, as given in table 5, show that the 82 to 98-day-old vaccines 7 to 11, which were prepared from the blood, liver, spleen, and kidney tissue of cockerels that had marked chicken-pox lesions and were killed on the tenth to the fourteenth day after inoculation, contained no virulent chicken-pox virus and possessed no immunizing properties. The vaccines of the same age prepared from lesion tissues of the same birds, as shown in table 5, did, however, contain living virus and immunizing properties, although the doses of these vaccines contained but from one-tenth to less than one-three-hundredth as much tissue as the doses of vaccines 7 to 11. These results tend to substantiate the observations made in the first tests of vaccines 7 to 11, that the immunity of vaccinated birds and the lesions on the inoculated birds might be due to accidental infection rather than to the vaccines. These results also suggest that the immunizing properties of preceding vaccines which contained both types of tissue were due entirely to their lesion-tissue content. For these reasons the inclusion of blood, liver, spleen, and kidney tissue in the experimental vaccines was discontinued.

TABLE 5

RESULTS OF THE SECOND IMMUNIZING AND VIRULENCE TESTS OF VACCINES 7 TO 16

Immunizing tests											Virulence tests[a]			
Vaccine				Number of fowl vaccinated	Number that developed vaccination-point lesions	Number that developed lesions on the head	Number that were tested for immunity 28 days after vaccination	Number that were immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Age of vaccine	Number inoculated	Lesions produced	Incubation period
No.	Type of tissue	Age *days*	Amount of tissue in dose *grams*								*days*			*days*
7	Blood and organs	98	0.33	4	0	0	3[b]	Marked lesions on 3	4	Marked lesions	86	6	None[c]	....
8	Blood and organs	97	.33	4	0	0	4	Marked lesions on 4	4	Marked lesions	85	6	None[c]	....
9	Blood and organs	96	.33	4	0	0	4	Marked lesions on 4	4	Marked lesions	84	6	None[c]	....
10	Blood and organs	95	.33	4	0	0	4	Marked lesions on 4	4	Marked lesions	83	6	None[c]	....
11	Blood and organs	94	.33	4	0	0	4	Marked lesions on 4	4	Marked lesions	82	6	None[c]	....
12	Lesion tissue	98	.03	4	0	0	4	Marked lesions on 3 Moderate lesions on 1	4	Marked lesions	86	6	Marked	5
13	Lesion tissue	97	.01	4	4	0	3[b]	3 immune	4	Marked lesions	85	6	Marked	5
14	Lesion tissue	96	.005	4	4	0	4	4 immune	4	Marked lesions	84	6	Marked	5
15	Lesion tissue	95	.002	4	4	0	4	4 immune	4	Marked lesions	83	6	Marked	5
16	Lesion tissue	94	0.001	4	3[d]	0	4	3 immune Mild lesions on 1[d]	4	Marked lesions	82	6	Marked	5

[a] The amount of tissue in 1 cc was the same as in the corresponding immunizing test.
[b] One died from causes not related to vaccination.
[c] Subsequent inoculation of these birds with chicken-pox virus showed all of them to be susceptible to the infection.
[d] The bird that did not develop vaccination lesions is the one that was not immune.

All of the thirty cockerels inoculated with the 82 to 86-day-old vaccines 12 to 16 developed marked chicken-pox lesions. In all instances, the lesions became visible after an incubation period of five days. No differences in the amount of virulence of the virus was detected.

In the immunizing tests with these vaccines, however, the results were not so uniform. None of the four birds that received vaccine No. 12 developed vaccination lesions or were completely immune to infection 28 days after vaccination, but all except one of the sixteen birds that received vaccines 13, 14, 15, or 16 developed lesions at the point of injection of the vaccine and were immunized. Since the virulence tests of these vaccines, which were started 12 days earlier, indicated that their immunizing properties should be approximately the same, the failure of vaccine No. 12 to immunize may be due to a decrease in the virulence of its virus content during the 12-day interval. The results of the third virulence test of these vaccines, which is described later, indicate that this explanation is probably correct.

As previously stated, fifteen of the sixteen birds that received a dose of vaccine No. 13, 14, 15, or 16, containing 0.01, 0.005, 0.002, or 0.001 gram of tissue developed vaccination-point lesions and became immunized to chicken-pox infection. The bird that did not become absolutely immune to chicken-pox was that one of the four that received a dose of vaccine No. 16, containing 0.001 gram of tissue, and that did not develop vaccination-point lesions. This is the first instance in the series of experiments to indicate that the occurrence of lesions at the point of vaccination may be essential for the development of complete immunity. The failure of one of the four birds vaccinated with vaccine No. 16 to become completely immunized to infection with chicken-pox virus cannot be said to definitely indicate that this vaccine was less effective than vaccines 13, 14, or 15; the difference in the results may have been due to the smaller amount (0.001 gram) of tissue in a dose of vaccine No. 16 than the amounts (0.01, 0.005, or 0.002 gram) of tissue in the other vaccines.

Third, Fourth, and Fifth Virulence and Immunizing Tests of Vaccines 12 to 16.—In these tests, dilutions of vaccines 12, 13, 14, 15, and 16 containing 0.05, 0.01, 0.005, 0.002, and 0.001 gram of tissue per cubic centimeter, respectively, were used. The virulence tests were made first. An immunity test of a vaccine was not made when the virulence test indicated that the virus in it was no longer virulent. The manner of making the tests was the same as in preceding experiments. Table 6 gives the age of the vaccines when the tests were made, the number of birds used, and the results obtained.

TABLE 6

RESULTS OF THIRD, FOURTH, AND FIFTH VIRULENCE AND IMMUNIZING TESTS OF VACCINES 12, 13, 14, 15, AND 16

Immunizing tests											Virulence tests			
Vaccine No.	Test No.	Vaccine: Age *days*	Vaccine: Amount of tissue in 1 cc *grams*	Number of fowls vaccinated	Number that developed vaccination-point lesions	Number that developed lesions on the head	Number tested for immunity 28 days after vaccination	Number immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Age of vaccine *days*	Number inoculated	Lesions produced	Incubation period *days*
12	3[a]	....	....	....	....	....	....	....	....	....	128	3	None	....
13	3	140	0.01	4	4	0	4	4 immune	4	Marked	127	3	Marked lesions on all	6
	4	184	.01	4	0	0	3[b]	1 immune Slight lesions on 2	2	Marked	184	2	Two tumors on each bird	7
	5	337	.1	3	0	0	3	Marked lesions on 3	2	Marked	320	2	None	....
	3	139	.005	4	4	0	4	4 immune	4	Marked	126	3	Marked lesions on all	6
	4	183	.005	4	4	0	2[c]	2 immune	2	Marked	183	2	Both marked lesions	7
14	5	336	.1	8	0	0	8	Marked lesions on 8	2	Marked	319	2	2 tumors on 1 bird 1 tumor on 1 bird	7

[a] Since the virulence test was negative, no immunizing tests with this vaccine were made.
[b] One died from causes not related to vaccination.
[c] Two died from causes not related to vaccination.
[d] Three died from causes not related to vaccination.
[e] One chicken-pox tumor developed on the wattle on the 28th day after vaccination.

TABLE 6—(*Continued*)

Immunizing tests											Virulence tests			
Vaccine No.	Test No.	Vaccine: Age *days*	Vaccine: Amount of tissue in 1 cc *grams*	Number of fowls vaccinated	Number that developed vaccination-point lesions	Number that developed lesions on the head	Number tested for immunity 28 days after vaccination	Number immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Age of vaccine *days*	Number inoculated	Lesions produced	Incubation period *days*
	3	138	.002	4	3	0	3[b]	3 immune	4	Marked	125	3	Marked lesions on all	6
15	4	182	.002	4	0	0	4	3 immune Slight lesions on 1	2	Marked	182	1	Marked lesions	7
	5	335	.1	8	0	0	5[d]	2 immune; marked lesions on 2 Slight lesions on 1	2	Marked	318	2	Moderate lesions on both	7
	3	137	.001	4	3	0	4	4 immune	4	Marked	124	3	Marked lesions on all	6
16	4	181	.001	4	2	1[c]	4	4 immune	2	Marked	181	2	1 tumor on each bird	7
	5	334	0.1	8	0	0	6[c]	4 immune Marked lesions on 1 Slight lesions on 1	2	Marked	317	2	6 tumors on 1 bird No lesions on 1 bird	7

[a] Since the virulence test was negative, no immunizing tests with this vaccine were made.
[b] One died from causes not related to vaccination.
[c] Two died from causes not related to vaccination.
[d] Three died from causes not related to vaccination.
[e] One chicken-pox tumor developed on the wattle on the 28th day after vaccination.

The third virulence test showed that the 128-day-old vaccine No. 12 no longer contained virulent chicken-pox virus. Therefore, no further tests of this vaccine were made.

The other four vaccines, Nos. 13, 14, 15, and 16, produced marked lesions on all of the birds used for the virulence tests and immunity of all of fifteen birds that were vaccinated and tested for immunity 28 days later. From these results it would appear, therefore, that these vaccines had not decreased in virulence or immunizing properties during 137 to 140 days of aging. Lesions developed at the point of vaccination on all but two of the sixteen birds vaccinated. Both of these birds, however, were immunized by the vaccine. This further demonstrates that the immunity to chicken-pox may be produced by vaccination without the production of visible lesions.

In the fourth test made when the vaccines were from 181 to 184 days old, vaccine No. 13, which contained the largest amount (0.01 gram) of tissue per cubic centimeter, caused but very slight lesions on the two cockerels used in the virulence test and absolute immunity of but one of three birds with which the immunizing tests was completed. The other two birds used in the latter test were resistant to infection but not absolutely immune. Vaccination lesions occurred on none of the birds. The lesions produced in the virulence test of vaccine No. 16, which contained the smallest amount (0.001 gram) of tissue per cubic centimeter, were no less pronounced than in the corresponding test of vaccine No. 13. All of four cockerels vaccinated with vaccine No. 16, however, became immunized. Vaccines 14 and 15, which contained 0.005 and 0.002 gram of tissue per cubic centimeter, respectively, produced marked lesions on the birds used in the virulence tests and immunity of five or six birds vaccinated. The lesions that developed on the bird that was not immune were of little consequence. Two other birds that were vaccinated were lost to the experiment by death.

These results are not entirely in harmony. Since vaccines 13 and 16 were found to contain but little virulent virus, while vaccines 14 and 15 possessed an abundance, it would be expected that vaccines 13 and 16 would be less effective immunizing agents than vaccines 14 and 15. This appeared to be true of vaccine No. 13 but vaccine No. 16 was more effective in this respect than vaccine No. 15 and just as effective as vaccine No. 14. Vaccines 14 and 15 appeared to be of equal virulence but the latter failed to immunize as consistently as the former. It would seem, therefore, that while the presence of virulent virus in vaccine is necessary for the production of immunity, the

extent of the lesions produced in virulence tests is not an absolute index of the immunizing property of vaccine.

The fifth virulence tests of vaccines 13, 14, 15, and 16, which were made when the vaccines were from 317 to 320 days old, indicated that no living virus remained in vaccine No. 13 and that the amount of living virus in the other vaccines was small. In the immunity tests, therefore, which were made 17 days later, instead of doses of vaccine varying in tissue content from 0.001 to 0.1 gram, the amount of tissue in a dose was 0.1 gram for all vaccines. None of eleven birds vaccinated with Nos. 13 and 14 were immunized. Six of eleven birds with which immunizing tests of vaccines 15 and 16 were completed became immune. The remaining five birds vaccinated with these two vaccines developed slight to marked lesions after inoculation with chicken-pox virus 28 days later. These results show that these vaccines had lost most of their virulence and immunizing properties and, therefore, no further work with them was done.

This series of experiments with ten vaccines, five of which (Nos. 7, 8, 9, 10, and 11) were prepared from the blood, liver, spleen, and kidneys, and five of which (Nos. 12, 13, 14, 15, and 16) were prepared from the lesion tissue has failed to show definitely that the vaccines prepared from the blood-and-organ tissue contained virulent virus or possessed immunizing properties. The lesion-tissue vaccines, however, were shown to contain an abundance of virulent virus and, when injected subcutaneously, were shown to be capable of establishing an immunity to artificial infection with chicken-pox virus within 28 days. The use of doses of vaccine containing amounts of tissue varying from 0.1 to 0.001 gram did not indicate that a dose containing the largest amount was more effective than one containing the smallest amount.

Forty-two of the sixty-five fowls that became immunized developed chicken-pox lesions at the point of injection after vaccination. The fowls that did not develop such lesions were principally from those vaccinated after the vaccine had aged 180 days or more. That the vaccination point of all immunized fowls did not become infected is further evidence that such infection is not essential to immunization. In this and all preceding experiments, however, it has been observed that all fowls that developed vaccination lesions did become immune. Such lesions have proved harmless, in so far as their effect on the general health of the fowls or tendency to initiate more widespread infection is concerned. Therefore, they may be regarded as a vaccination 'take' indicating that immunization will be accomplished, and as a favorable reaction to vaccination with fresh-lesion-tissue vaccine.

As previously stated, the purpose of securing material for the preparation of these vaccines from cockerels that were killed on the tenth, eleventh, twelfth, thirteenth, and fourteenth days, respectively, after inoculation with chicken-pox virus, was to obtain data regarding the most suitable time after inoculation to secure tissue for vaccine preparation. From the standpoint of yield of tissue per bird the 12-day lesion rank first, the 13, 14, 11 and 10-day lesions following in the order named.

In the tests of the fresh vaccines, no differences between them were detected. The tests made when the vaccines were from 94 to 98 days old indicated that vaccine No. 12, prepared from 10-day lesions, had decreased considerably in immuzing properties. The other four vaccines were apparently unchanged. The third test, made when the vaccines were from 127 to 140 days old, showed that vaccine No. 12 was entirely impotent, while immunizing properties of the others were apparently undiminished. At the fourth test, made when the vaccines were 181 to 184 days old, it was found that vaccine No. 13, prepared from 11-day lesions, had lost most of its virulence and ability to immunize, but that the other three were still active in these respects. The fifth test was made when the vaccines were from 334 to 337 days old. Vaccine No. 13 had lost all of its virulence and immunizing properties. Vaccines 14, 15, and 16 still contained some virulent virus but were poor immunizing agents. The vaccines prepared from chicken-pox lesions removed on the twelfth, thirteenth, and fourteenth days after inoculation were shown to retain their virulence and immunizing property longer than those prepared from lesions of cockerels killed 10 or 11 days after inoculation. The cockerels killed on the twelfth, thirteenth, and fourteenth days after inoculation also, as shown previously, yielded more lesion tissue than those killed earlier. These results, therefore, suggest that the most suitable time for securing material for the preparation of fresh lesion tissue vaccine is on the day that the lesions have attained maximum development, or one or two days after. When highly virulent virus is used for inoculation, the time between inoculation and the proper time for removal of the lesions would probably not vary more than one or two days, from the 12 to 14-day period of these experiments.

EXPERIMENTS WITH VACCINES PREPARED FROM LESION TISSUE

These experiments consist of immunizing and virulence tests of a series of five vaccines (Nos. 20, 21, 23, 24, and 25) prepared from fresh lesion tissue in the same manner as vaccines 12 to 16 in the preceding experiment. A brief description of the vaccines follows.

Vaccines 20 and 21 were prepared on March 29 and April 28, 1926, respectively, from lesions removed from cockerels on the twelfth day after vaccination. Each cubic centimeter of these vaccines contained 0.1 gram of tissue. Most of the immunizing and virulence tests of these vaccines were made with dilutions containing 0.005, 0.002, and 0.001 gram of tissue per cubic centimeter. The dilutions were always made immediately before use. Virulence tests were made of vaccine No. 20 when it was 15, 42, 51, 61, 71, and 219 days old. Immunizing tests were made when it was 29, 51, 66, and 236 days old. The virulence tests of vaccine No. 21 were made when it was 13, 55, and 219 days old, and the immunizing tests when it was 55 and 236 days old.

Vaccines 23, 24, and 25 were prepared from 12-day lesions on June 14, June 24, and August 31, 1926, respectively. Each cubic centimeter of these vaccines contained 0.25 gram of tissue. The virulence and immunizing tests were mainly of dilutions containing 0.005, 0.002, or 0.001 gram of tissue per cubic centimeter. Vaccine No. 23 was tested for virulence when it was fresh and 36 days and 142 days old, and for immunizing properties when it was fresh and 161 days old. Virulence tests of vaccine No. 24 were made with 86 and 182-day-old vaccine. Virulence tests of vaccine No. 25 were made on the seventh, thirtieth, and sixty-fourth days, and immunity tests on the seventh, thirtieth, and seventy-first days after its preparation.

The number of birds used and other details regarding the tests are given in table 7.

As shown in table 7, there was so little difference between the results of the immunizing and virulence tests of the five vaccines that they may be discussed as a whole instead of separately.

The virulence tests may be grouped as those with vaccine from 1 to 75 days old and as those with vaccines from 132 to 219 days old. No tests were made with vaccines between the ages of 75 and 132 days.

TABLE 7

Results of the Immunizing and Virulence Tests of Vaccines 20, 21, 23, 24, and 25

Immunizing tests									Virulence tests					
Vaccine No.	Test No.	Vaccine: Age *days*	Vaccine: Amount of tissue in 1 cc *grams*	Number of fowls vaccinated	Number that developed lesions at vaccination-point	Number that developed lesions on the head	Number tested for immunity	Days after vaccination when tested	Number immune or that developed chicken-pox lesions	Test No.	Age of vaccine *days*	Tissue in 1 cc of vaccine *grams*	Number inoculated	Lesions produced	Incubation period *days*
20	1	29	0.005	4	4	0	4	28	4 immune	1	15	0.01	3	Marked	5
			.002	4	4	0	4	28	4 immune			.005	3	Marked	5
			.001	4	3	0	4	28	4 immune			.002	3	Marked	5
												.001	3	Marked	5
20	2	51	.005	4	3	0	4	28	4 immune	2	43	.005	2	Marked	6–7
			.002	4	4	0	4	28	4 immune			.002	2	Marked	6–7
			.001	4	3	0	4	28	4 immune			.001	2	Marked	6
20	3	66	.002	125	97	0	125	28	117 immune 8[a] slight lesions	3	51	.005 .002	2 2	Marked Marked[b]	7 8
												.001	2	Marked	8
20	4	236	.1	4	0	0	4	62	4 marked lesions	4	61	.005 .002	2 2	Marked Marked	7 7
												.001	2	Marked	7
										5	71	.005 .002	2 2	Marked Marked	7 7
												.001	2	Marked	7
										6	219	.1	2	None	....

[a] Four of these had vaccination-point lesions—four had not. All lesions healed in 15 days after inoculation.
[b] Lesions not as marked as those on birds inoculated with .002 gram dilution.
[c] Eight died from infectious bronchitis.
[d] One died from infectious bronchitis.
[e] Three died from undetermined cause.
[f] One of these had vaccination-point lesions—one had not. All lesions healed within 18 days after inoculation.
[g] Lesions healed in from 12 to 14 days after inoculation.

TABLE 7—(*Continued*)

Immunizing tests									Virulence tests					
Vaccine No.	Test No.	Vaccine: Age *days*	Vaccine: Amount of tissue in 1 cc *grams*	Number of fowls vaccinated	Number that developed lesions at vaccination-point	Number that developed lesions on the head	Number tested for immunity	Days after vaccination when tested	Number immune or that developed chicken-pox lesions	Test No.	Age of vaccine *days*	Tissue in 1 cc of vaccine *grams*	Number inoculated	Lesions produced	Incubation period *days*
21	1	55	.005	4	4	0	4	28	4 immune			.005	2	Marked	6
			.002	10	8	0	2[c]	28	2 immune	1	13	.002	2	Marked	6
			.001	4	4	0	3[d]	28	3 immune			.001	2	Marked	6
												.005	2	Marked	7
										2	55	.002	2	Marked	7
												.001	2	Marked	7
			.1	4	0	0	3[d]	62	3 immune			.1	2	No lesions on one	12
21	2	206	.005	4	0	0	4	62	4 immune					6 tumors on one	
			.002	4	0	0	3	62	3 immune			.005	2	None	....
			.001	4	0	0	3	62	3 immune	3	189	.002	2	No lesions on one	12
														One tumor on one	
												.001	2	No lesions on one	12
														One tumor on one	
23	1	Fresh	.005	10	7[e]	0	7[e]	28	7 immune			.005	2	Marked	6
			.002	10	9	0	10	28	10 immune	1	Fresh	.002	2	Marked	6
			.001	10	9	0	7[e]	28	5 immune 2 slight lesions[f]			.001	2	Marked	6

[a] Four of these had vaccination-point lesions—four had not. All lesions healed in 15 days after inoculation.
[b] Lesions not as marked as those on birds inoculated with .002 gram dilution.
[c] Eight died from infectious bronchitis.
[d] One died from infectious bronchitis.
[e] Three died from undetermined cause.
[f] One of these had vaccination-point lesions—one had not. All lesions healed within 18 days after inoculation.
[g] Lesions healed in from 12 to 14 days after inoculation.

TABLE 7—(*Continued*)

Immunizing tests									Virulence tests					
Vaccine No.	Test No.	Vaccine: Age *days*	Vaccine: Amount of tissue in 1 cc *grams*	Number of fowls vaccinated	Number that developed lesions at vaccination-point	Number that developed lesions on the head	Number tested for immunity	Days after vaccination when tested	Number immune or that developed chicken-pox lesions	Test No.	Age of vaccine *days*	Tissue in 1 cc of vaccine *grams*	Number inoculated	Lesions produced	Incubation period *days*
										2	36	.002	2	Marked	7
			.005	4	0	0	3[d]	62	2 immune			.25	2	Marked	7
									1 slight lesion[g]			.005	2	Marked	7
23	2	161	.002	4	0	0	4	62	4 slight lesions[g]	3	142	.002	2	Moderate lesions on 1	7
			.001	4	0	0	4	62	3 immune					6 tumors on one	
									1 slight lesion[g]			.001	2	No lesions on one	7
														Moderate lesions on 1	
												.005	2	Marked	6
	1	86	.002	3	1	0	3	32	3 immune	1	75	.002	2	Marked	6
												.001	2	Marked	6
			.005	4	0	0	4	62	2 immune			.25	2	Marked	6
									2 slight lesions[g]			.005	2	Marked	6
24	2	151	.002	4	0	0	4	62	4 immune	2	132	.002	2	Marked lesion on one	7
			.001	4	0	0	4	62	3 immune					One tumor on one	
									1 slight lesion[g]			.001	2	No lesions on one	7
														Three tumors on one	

[a] Four of these had vaccination-point lesions—four had not. All lesions healed in 15 days after inoculation.
[b] Lesions not as marked as those on birds inoculated with .002 gram dilution.
[c] Eight died from infectious bronchitis.
[d] One died from infectious bronchitis.
[e] Three died from undetermined cause.
[f] One of these had vaccination-point lesions—one had not. All lesions healed within 18 days after inoculation.
[g] Lesions healed in from 12 to 14 days after inoculation.

TABLE 7—(*Concluded*)

Immunizing tests									Virulence tests					
Vaccine No.	Test No.	Vaccine: Age *days*	Vaccine: Amount of tissue in 1 cc *grams*	Number of fowls vaccinated	Number that developed lesions at vaccination-point	Number that developed lesions on the head	Number tested for immunity	Days after vaccination when tested	Number immune or that developed chicken-pox lesions	Test No.	Age of vaccine *days*	Tissue in 1 cc of vaccine *grams*	Number inoculated	Lesions produced	Incubation period *days*
25	1	7	.005	4	4	0	4	30	4 immune	1	7	.005	2	Marked	6
			.002	4	4	0	4	30	4 immune			.002	2	Marked	6
			.001	4	4	0	4	30	4 immune			.001	2	Marked	6
25	2	30	.005	4	4	0	4	28	4 immune	2	30	.005	2	Marked	6
			.002	4	4	0	4	28	4 immune			.002	2	Marked	6
			.001	4	4	0	4	28	4 immune			.001	2	Marked	6
25	3	71	.005	4	4	0	4	32	4 immune	3	64	.005	2	Marked	6
			.002	4	4	0	4	32	4 immune			.002	2	Marked	6
			0.001	4	4	0	4	32	4 immune			0.001	2	Marked	6

[a] Four of these had vaccination-point lesions—four had not. All lesions healed in 15 days after inoculation.
[b] Lesions not as marked as those on birds inoculated with .002 gram dilution.
[c] Eight died from infectious bronchitis.
[d] One died from infectious bronchitis.
[e] Three died from undetermined cause.
[f] One of these had vaccination-point lesions—one had not. All lesions healed within 18 days after inoculation.
[g] Lesions healed in from 12 to 14 days after inoculation.

Eighty birds were used in virulence tests of 1 to 75-day-old vaccine. Three were inoculated with vaccine containing 0.01 gram of tissue per cubic centimeter, twenty-five with vaccine containing 0.005 gram of tissue per cubic centimeter; twenty-seven with vaccine containing 0.002 gram of tissue per cubic centimeter, and twenty-five with vaccine containing 0.001 gram of tissue per cubic centimeter. In one instance, that of the two birds inoculated with 51-day-old vaccine, No. 20, the lesions produced by inoculation with the 0.001-gram dilution were less pronounced than those on the birds inoculated with the dilutions containing more tissue. Otherwise the lesions appeared to be of equal severity irrespective of the tissue content of the vaccines. The lesions appeared after an incubation period of from 5 to 8 days and were evenly distributed over the scarified area. By the twelfth or fourteenth day the lesions were very pronounced and covered an area one and one-half to two times as large as that scarified. The incubation period showed a tendency to increase slightly with the age of the vaccine. With vaccine No. 20, the increase was from 5 days for 15-day old vaccine to 8 days for 51-day-old vaccine and 7 days for 61-day-old vaccine. With vaccine No. 21, the incubation period was 6 days for fresh vaccine and 7 days for 55-day-old vaccine. With 7, 30, and 64-day-old vaccine No. 25, the incubation period was uniformly 6 days. Since the incubation period did not in all cases increase as the vaccine increased in age, it would seem just as probable that the slight differences in periods of incubation, after inoculation with the 1 to 75-day-old vaccine, were due to differences in the susceptibility of the birds as they were due to changes in the virus.

In contrast to the uniform results of virulence tests of different dilutions of the 1 to 75-day-old vaccine, the different dilutions of the 132 to 219-day-old vaccines did not produce lesions of the same degree of severity. Inoculation with dilutions of these vaccines containing 0.25 and 0.005 gram of tissue resulted in the development of pronounced lesions. The birds inoculated with dilutions containing 0.002 or 0.001 gram of tissue, however, developed either no lesions at all or only a few discrete tumors. These separate tumors were as persistent and became proportionately as pronounced as the more extensive lesions. The period of incubation was in all instances 6 or 7 days. It would seem, therefore, that the change that had occurred in the virus content as a result of aging was more probably a decrease in amount due to the death of part of the virus than to a decrease in virulence.

Inoculation with dilutions of 189-day-old vaccine containing 0.1, 0.005, 0.002, or 0.001 gram of tissue per cubic centimeter produced

either no lesions or a few discrete tumors. The period of incubation was 12 days. These results indicate that the virus in this vaccine had decreased in both amount and virulence.

Inoculation with 219-day-old vaccine containing 0.1 gram of tissue per cubic centimeter produced no lesions. From this it was concluded that all of the virus in the vaccine had been destroyed.

The immunizing tests may be divided into those of vaccines from 1 day to 86 days old and those of vaccines after they were from 151 to 236 days old. No tests were made with vaccines between 86 and 151 days old.

Immunizing tests of 1 to 86-day-old vaccines were completed with 221 birds. The tissue content of a dose of vaccine was 0.005, 0.002, or 0.001 gram. The results are summarized in table 8.

TABLE 8

SUMMARY OF RESULTS OF IMMUNIZING TESTS WITH 1 TO 86-DAY-OLD VACCINES 20, 21, 23, 24, AND 25

Grams of tissue in dose of vaccine	Number of fowls vaccinated	Number that developed vaccination-point lesions	Number that were immune
0.005	31	30	31
0.002	160	129	152
0.001	30	27	28
Totals.....	221	186	211

Table 8 shows that thirty-five of the 221 fowls that were vaccinated failed to develop a vaccination lesion or 'take.' The distribution of these birds is as follows:

1 was vaccinated with the 0.001 gram dilution of 29-day-old vaccine No. 20
1 was vaccinated with the 0.005 gram dilution of 51-day-old vaccine No. 20
1 was vaccinated with the 0.001 gram dilution of 51-day-old vaccine No. 20
28 were vaccinated with the 0.002 gram dilution of 66-day-old vaccine No. 20
1 was vaccinated with the 0.002 gram dilution of fresh vaccine No. 23
1 was vaccinated with the 0.001 gram dilution of fresh vaccine No. 23
2 were vaccinated with the 0.002 gram dilution of 86-day old vaccine No. 24

This shows that the absence of vaccination lesions was not confined to any vaccine or to any age or dilution of vaccine. That there were more failures among the birds vaccinated with the 0.002-gram dilution of vaccine No. 20 may be explained by the fact that this dilution of vaccine No. 20 was given to more than three-fourths of the birds vaccinated.

The lesions of the ten birds which, as shown in table 8, were not entirely immunized consisted of the formation of a small amount of dry yellow scab over the scarified area. These lesions never had the appearance of an active chicken-pox lesion and were entirely healed in from 12 to 18 days after inoculation. The birds were eight of the one hundred and twenty-five that were vaccinated with the 0.002-gram dilution of the 66 day-old vaccine No. 20 and two of the seven birds that were vaccinated with the 0.001-gram dilution of fresh vaccine No. 23. These results cannot be interpreted as indicating that vaccine No. 20 was less effective than the others, since this vaccine was used on more than three-fourths of the birds. If the other vaccines had been used on an equal number of birds it is probable that some failures to confer complete immunity would have been encountered.

The ten birds that were not absolutely immunized were equally distributed among those that developed vaccination-point lesions and those that did not. This again demonstrates that immunity can develop in the absence of a lesion at the point of vaccination or other visible vaccination reaction. It also demonstrates that the occurrence of a lesion at the point of vaccination is not a positive indication of the development of complete immunity. It should be pointed out, however, that the number of the birds that were not entirely immune was proportionately greater among those that did not develop vaccination lesions than among those that did develop such lesions.

None of the forty birds with which, as shown in table 7, the immunizing tests of the 151 to 236-day-old vaccine were completed, developed vaccination lesions. Inoculation with chicken-pox virus sixty-two days after vaccination showed that fourteen of the twenty-three birds that received 151 or 161-day-old vaccine and all of ten birds that received 206-day-old vaccine were immune. The character of the lesions that were produced on the nine birds that were not immune indicated that they had a high degree of resistance to the infection. These results show that the 151 to 206-day-old vaccines were less efficient immunizing agents than they had previously been and are in agreement with the results of the virulence tests, which showed that the virus content of the vaccines had become reduced during the period of storage. The four birds vaccinated with the 236-day-old vaccine acquired neither immunity nor increased resistance to infection with chicken-pox virus. These results are in accordance with the virulence tests, which showed that the vaccine contained no virulent chicken-pox virus.

SUMMARY AND DISCUSSION OF THE RESULTS OF THE VACCINATION EXPERIMENTS

In the preliminary experiments it was demonstrated that by subcutaneous injections with vaccine prepared from the whole comb of cockerels with extensive chicken-pox lesions, fowls can be immunized against artificial infection with chicken-pox virus 28 days later. Equally good results were obtained with vaccine prepared from the whole comb, blood, liver, spleen, and kidneys of infected cockerels. Both of the above types of vaccine were shown to contain a virulent chicken-pox virus, but further comparative experiments with a vaccine prepared with comb tissue and one prepared with the blood, liver, spleen, and kidney tissue of infected cockerels failed to demonstrate definitely that the latter type of vaccine possessed immunizing properties. The use of blood, liver, spleen, and kidney tissue in the experimental vaccines was therefore discontinued. The vaccine containing comb tissue retained its potency for as long as 86 days. One dose was as effective as two doses given 7 days apart. Small chicken-pox lesions developed on the skin of many fowls at the point where the vaccine was injected. These lesions did not spread to other parts nor otherwise prove harmful to the birds.

The fibrous portion of the comb tissue was with difficulty reduced to a fine state, much of it was removed by straining, and that portion of it remaining in the vaccine was thought to be inert. Therefore the plan was adopted of using only the lesion and sub-lesion tissue instead of the entire comb. Since this type of vaccine was considered more satisfactory than the preceding, the results obtained with it will be discussed in more detail. The lesion-tissue vaccines as prepared contained either 0.1 or 0.25 gram of tissue per cubic centimeter. The volume of a dose of vaccine was uniformly 1 cc. to each bird. Variation in the size of dose was accomplished by making dilutions of the original preparation containing different amounts of tissue per cubic centimeter. The dilutions that were used most extensively contained 0.005, 0.002, and 0.001 gram of tissue in a cubic centimeter. The age of the vaccines at the time of use varied from 1 day to 337 days. Because of the similarity of the results obtained with vaccines between certain age limits, it is possible to summarize the results as those obtained with vaccines from 1 day to 140 days old and those obtained with vaccines from 151 to 337 days old. Such a summary is given in table 9.

TABLE 9

SUMMARY OF RESULTS OF VACCINATION WITH LESION-TISSUE VACCINE

Age of vaccine *days*	Number of fowls vaccinated	Number immunized	Per cent immunized	Fowls that developed vaccination lesions				Fowls that did not develop vaccination lesions			
				Total number	Per cent of number vaccinated	Number immune	Per cent immune	Total number	Per cent of number vaccinated	Number immune	Per cent immune
1 to 140	275	263	95.6	234	85.0	229	97.8	41	14.9	34	82.9
151 to 337	74	43	58 1	4	5.4	4	100.0	70	94.5	39	55.7

As shown in table 9, the 1 to 140-day-old vaccines were highly efficient immunizing agents and caused the development of lesions at the point of vaccination on 85 per cent of the fowls. Thirty-four of the immune fowls were among those that did not develop vaccination lesions. This indicates that a vaccination lesion was not essential to the development of complete immunity. It is seen, however, that the percentage of fowls that were immunized is higher among those that developed vaccination lesions than among those that did not. This shows that a vaccination lesion may be regarded as an indication of the development of immunity and, therefore, as a favorable reaction after vaccination.

The inoculation of cockerels with the same dilutions of the 1 to 140-day-old vaccines as were used for vaccination produced marked chicken-pox lesions on the scarified comb surfaces. As previously shown, this vaccine, when injected subcutaneously, proved innocuous except for the production of slight lesions at the point of injection. This shows that the subcutaneous injection of birds with vaccine containing highly virulent virus is unlikely to produce harmful lesions. This is a factor of great importance in the production of chicken-pox vaccine such as is used in these experiments, since the immunizing property of such vaccine is dependent upon its virus content. The fact that vaccinated birds developed vaccination-point lesions indicate that they can transmit infection to susceptible fowls with which they might be associated. In the use of such vaccine on a poultry farm, therefore, it would be necessary that all susceptible fowls be vaccinated.

The results obtained with the 151 to 337-day-old vaccine, as shown in table 9, require little comment. Of the seventy-four fowls vaccinated but forty-three were immunized and only four developed vaccination-point lesions. These results indicate that the 151 to 337-day-old vaccines were less satisfactory immunizing agents than when they

were from 1 to 140 days old. The virulence tests of these vaccines demonstrated that the amount of virus in all vaccines had been reduced during the aging periods and in some cases had been entirely destroyed.

THE LONGEVITY OF VIRULENCE OF VIRUS IN LESION-TISSUE VACCINE AS INFLUENCED BY THE PROPORTION OF TISSUE AND DILUENT

A vaccine was prepared from such amounts of lesion tissue and glycerine-phenolized-saline mixture as to make 1 cc. of the final product contain 0.25 gram of tissue. Seven dilutions of this vaccine with the glycerine-phenolized-saline mixture were immediately made. The seven dilutions contained 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, and 0.001 gram of tissue, respectively, in one cubic centimeter of vaccine.

TABLE 10

THE RESULTS OF TESTS TO SHOW THE EFFECT OF AGE UPON THE VIRULENCE OF THE VIRUS IN DILUTIONS OF VACCINE CONTAINING DIFFERENT AMOUNTS OF TISSUE PER CUBIC CENTIMETER

Test number	Age of vaccine *days*	Age of dilution *days*	Grams of tissue in one cc	Number inoculated	Lesions produced
2	31	31	0.25	2	Marked
		31	.001	2	None
3	40	40	.25	2	Marked
		40	.10	2	Marked
		40	.05	2	Marked
		40	.02	2	Marked
		40	.01	2	None
		40	.005	2	None
		40	.002	2	None
		40	.001	2	None
4	50	Fresh	.01	2	Marked
		Fresh	.005	2	Marked
		Fresh	.002	2	Marked
		Fresh	.001	2	Marked
5	177	177	.25	2	Marked
		177	.1	2	2-4 tumors on each bird
		177	.05	2	None
		177	.02	2	None
		Fresh	.1	2	Marked
		Fresh	.05	2	Moderate
		Fresh	.02	2	3 tumors on each bird
		Fresh	.01	2	1 tumor on each bird
		Fresh	.005	2	1 tumor on each bird
		Fresh	.002	2	2 tumors on each bird
		Fresh	0.001	2	None

These preparations were stored in an icebox and tested at intervals for the presence in them of virulent virus. The tests consisted of applying vaccine to a scarified surface of the combs of cockerels. Chicken-pox lesions were produced when the vaccine contained virulent virus. The first tests were made with fresh preparations and showed that each dilution contained an abundance of virulent virus. Other tests were made when the vaccine and dilutions were 31, 40, 50, and 177 days old. The results are given in table 10.

As shown in table 10, the results of the second virulence tests, which included only dilutions containing the highest (0.25 gram) and lowest (0.001 gram) concentrations of tissue, indicated that the 0.25-gram concentration contained virus of unimpaired virulence while that of the virus in the 0.001-gram dilutions was entirely lost during the 31 days of storage. These results led to the testing of all of the dilutions of vaccine at the age of 40 days. The results showed that virulent virus was still abundant in the dilutions containing 0.25, 0.1, 0.05, 0.02 gram of tissue per cubic centimeter, but failed to demonstrate the presence of virulent virus in the dilution containing 0.01, 0.005, 0.002, or 0.001 gram of tissue per cubic centimeter. Fresh dilutions containing these latter amounts of tissue per cubic centimeter were then made from the 0.25-gram dilutions and tested for virulence. Marked chicken-pox lesions were produced in all instances. This demonstrated that the negative results of the virulence test of the 40-day-old dilutions were due to destruction of the virus during the period of storage. Therefore no further tests of the dilutions of vaccine containing 0.01, 0.005, 0.002, or 0.001 gram of tissue per cubic centimeter were made. The results of virulence tests that were made when the vaccine was 177 days old indicated that in the 0.02 and 0.05-gram dilution there remained no virulent virus, and in the 0.1-gram dilution but a small amount. The undiluted (0.25-gram) vaccine was found still to contain virulent virus. The results of virulence tests of fresh dilutions of this vaccine, however, indicated that the amount of virus in the undiluted vaccine was much less than it had been previously.

The results of this series of tests indicate that the longevity of the virulence of chicken-pox virus in fresh lesion-tissue vaccine decreases as the proportion of tissue to diluent decreases. It is therefore desirable, in the preparation of vaccine that is to be stored, to use only as much diluent as is necessary to facilitate the grinding process and to destroy contaminating bacteria. Dilutions that are used for vaccination should be made immediately before the vaccine is administered.

RESULTS OF THE USE OF LESION-TISSUE VACCINE IN INFECTED FLOCKS

The preceding experiments have demonstrtaed that there is apparently little danger of inducing harmful chicken-pox infection by the subcutaneous injection of vaccine containing virulent chicken-pox virus. All of the birds used in these experiments were healthy cockerels and were kept under good environmental conditions apart from other fowls. It was not demonstrated, therefore, that such vaccine could with safety be administered to flocks in which chicken-pox existed or in which other diseases were present, or during periods of cold, wet, or otherwise unfavorable weather. It was thought that the use of vaccine containing virulent virus under such conditions might increase the severity of the disease in infected birds or in birds that were in the incubation stage of disease at the time of vaccination, or result in extensive infection with chicken-pox among those birds that were free from infection at the time of vaccination. To secure information on these points vaccine was administered to 3125 fowls in four flocks in which some of the fowls were infected with chicken-pox. In flock No. 1, the infection was produced by adding a few diseased birds to a flock of cockerels. These were kept in the houses that were used for experimental purposes. The other three flocks were farm flocks in which chicken-pox from natural infection was occurring. The results follow:

Flock No. 1.—One hundred and ninety-five healthy cockerels were confined with cockerels infected with chicken-pox until fifty-one, or 26.1 per cent, of the healthy had developed chicken-pox lesions. Seventy-six of the healthy birds were then vaccinated with a dose of vaccine containing 0.002 gram of tissue. Tests showed that the vaccine contained an abundance of virulent chicken-pox virus. Sixty-eight of the healthy birds were not vaccinated. During the two weeks after vaccination, lesions appeared about the head of twenty-five, or 32.8 per cent, of those that were vaccinated, and twenty-nine, or 52.6 per cent, of those that were not vaccinated. No additional fowls in either group became infected. Lesions developed at the point of vaccination on thirty-nine, or 51.3 per cent, of the seventy-six birds vaccinated. Only nine of these were among the twenty-five vaccinated fowls that developed lesions about the head. This indicates that there was no relation between the occurrence of lesions at the point of vaccination and the occurrence of them about the heads of the fowls.

Lesions on the head did not become severe enough to cause death of any of the birds. All that became infected had recovered within 36 days after vaccination. It is evident, therefore, that neither the rate of spread of the infection among the birds nor the severity of the lesions on those that became infected was increased by vaccination.

Vaccine was administered to twenty-six of the fifty-one birds that had become infected before any of the healthy birds were vaccinated. The lesions on those vaccinated did not become any more severe and were no more persistent than the lesions on those that were not vaccinated.

Flock No. 2.—The second flock consisted of 858 hens of the White Leghorn, Rhode Island Red, and Barred Plymouth Rock breeds. They were vaccinated with vaccine containing 0.002 gram of tissue per cubic centimeter. It was demonstrated by tests that the vaccine contained an abundance of virulent virus. Seventeen fowls had become infected prior to vaccination. There were two additional cases of chicken-pox in the flock after vaccination, the last case developing on the twenty-third day. None of the fowls died from this cause. During this period infectious bronchitis became prevalent, causing a sickness of approximately one-third and the death of about 7 per cent of the fowls. The weather at the time of and after vaccination was generally cold and damp.

Flock No. 3.—The third flock consisted of approximately 1,700 White Leghorn pullets. The dose of vaccine given contained 0.002 gram of tissue. The presence of virulent virus in the vaccine was determined by virulence tests. The vaccine was administered and all observations of results were made by the owner of the flock. Detailed data regarding the condition of this flock were not obtained. It is known, however, that many of the birds were infected with chicken-pox and that losses were occurring from infectious bronchitis and ruptured yolk. The weather was cold and wet. The owner stated that the loss from chicken-pox was slight and that all evidence of that disease disappeared in about three weeks after vaccination.

Flock No. 4.—The fourth flock consisted of 372 White Leghorn hens, forty-nine of which had chicken-pox lesions. Two hundred of the healthy birds were vaccinated and one hundred and fifteen were left unvaccinated for controls. After vaccination, thirty-three, or 16.5 per cent, of the vaccinated group and thirty-six, or 31.3 per cent, of the control group developed the disease. The last case of chicken-pox in the control group occurred on the twenty-eighth day after vaccination and all except three of the thirty-three cases in this group

occurred before the seventeenth day after vaccination. In the control group, thirteen of the thirty-six cases of chicken-pox occurred after the twenty-eighth day and seventeen of the cases after the seventeenth day after the date of vaccination.

From the results obtained with these four flocks it is seen that the administration of vaccine containing virulent chicken-pox virus to flocks in which chicken-pox or other diseases were present and when cold and wet weather prevailed had no harmful effects.

FIELD TRIALS WITH VACCINE PREPARED FROM FRESH LESION TISSUE

These trials were carried out on poultry farms on which chicken-pox had been prevalent in the past but on which little or no active infection existed at the time the trials were instituted. They consisted in the vaccination of the pullets and cockerels when they were from four and one-half to six months old and before any of them had become infected with chicken-pox. After vaccination individual examinations of the fowls were made to detect those on which chicken-pox lesions developed either at the point of injection of the vaccine or about the head. In these trials it was possible to leave only a few birds unvaccinated as controls, since they were carried out on poultry farms and the owners were unwilling to risk the loss incident to infection that might occur among the fowls that were not vaccinated.

Virulence and immunizing tests of each lot of vaccine used in the field trials were made at the laboratory. By these means it was determined that all vaccines used in the field trials possessed an abundance of virulent virus and would immunize fowls against artificial infection with chicken-pox virus 28 days after vaccination. Five different vaccines, Nos. 20, 21, 23, 24, and 25, were used. They were prepared in an identical manner but varied with respect to their tissue content, Nos. 20 and 21 containing 0.1 gram of tissue per cubic centimeter, and Nos. 23, 24, and 25 containing 0.25 gram of tissue per cubic centimeter. The vaccine was diluted sufficiently at the time of use so that the dose was uniformly 1 cc. containing 0.002 gram of tissue. The age of the vaccines at the time of use varied from 31 to 85 days.

The primary purpose of the field trials was to determine if young healthy fowls on poultry farms could be vaccinated with lesion-tissue vaccine containing virulent chicken-pox virus without danger of producing serious chicken-pox infection among them. It was hoped that information might also be obtained regarding the extent to which the

fowls were protected against natural infection during the winter period after vaccination. It was realized, however, that, since the trials were uncontrolled, failure of chicken-pox to occur could not with certainty be attributed to protection afforded by vaccination, even though chicken-pox virus was undoubtedly present on the premises on which the fowls were kept. An additional feature of the field trials was to obtain information regarding the length of immunity produced by vaccination. For this purpose fowls were secured at varying intervals after vaccination, their combs scarified, and chicken-pox virus applied to the scarified surface.

The description and results of the field trials follow:

Field Trial No. 1.—On the farm at which field trial No. 1 was conducted chicken-pox had been continuously present for more than a year. At the time this trial was started (April 30, 1926) the disease was confined to a few hens in one small laying house, and to a few of a lot of 600 two to three-month-old pullets and cockerels. All of the infected and contact birds were well isolated from the remainder of the flock and the outlook was that the spread of the disease had been checked for the time. All of the young stock was reared on clean range at a considerable distance from the rest of the flock. However, when they reached the age of from four to five months they were transferred to laying houses and yards that had recently been occupied by infected flocks. The probability that some of the birds would become infected from contact with chicken-pox virus in the laying houses was therefore great.

The plan of vaccinating the birds as they were moved from the range to the laying houses was adopted. Since the birds were hatched at various times from January to April, they were moved to the laying houses in twelve lots between April 30 and September 30. Slightly more than half of the first lot of birds were left unvaccinated. All of the birds in the other lots were vaccinated. At least two examinations of each bird were made during the first four weeks after vaccination. The total number of birds vaccinated was 9,361. By means of colored leg bands the birds were marked so that those that developed vaccination lesions could later be distinguished from those that did not. The colored bands also served to identify the birds according to the lot to which they belonged and the date upon which they were vaccinated.

Field Trial No. 2.—The second field trial was carried out on a poultry farm on which an outbreak of chicken-pox had occurred during each winter for the last five years. There were no infected birds on the premises when this trial was started (September 17,

1926). All of the pullets and cockerels that were hatched during 1926 were vaccinated. They consisted of 799 White Leghorn and Barred Plymouth Rock cockerels from three to seven months old, 188 White Leghorn and Barred Plymouth Rock pullets from four to five months old, 340 White Leghorn pullets from six to seven months old, and 205 Barred Plymouth Rock pullets from six to seven months old. The weight of the birds varied from less than two pounds in the case of some of the young Leghorn pullets and cockerels to seven or eight pounds in the case of some of the Rock cockerels. The dose of vaccine, however, was uniformly 1 cc. containing 0.002 gram of tissue. The six to seven-month-old Leghorn and Rock pullets were producing approximately sixty eggs for each one hundred birds daily at the time of vaccination.

The results of the field trials are summarized in table 11.

TABLE 11

RESULTS OF FIELD TRIALS OF VACCINE PREPARED FROM FRESH LESION TISSUE

Birds were White Leghorns from four to five months old unless otherwise noted. Dose—1 cc. containing 0.002 gram tissue.

Lot No.[a]	Vaccine		Number vaccinated	Birds that developed lesions at point of vaccination		Birds that developed lesions on the head	
	No.	Age *days*		Number	Per cent	Number	Per cent
1[b]	20[c]	31	250 ♀	250	100.0	12	4.8
2	20	50	726 ♀	719	99.1	50	6.8
3	20	58	586 ♀	433	73.9	20	3.5
4	20	67	514 ♀	227	44.1	0	0.0
5	20	79	405 ♀	105	25.9	0	0.0
6	20	82	667 ♂	40	6.1	0	0.0
7	21[c]	58	755 ♀	143	18.9	5	0.6
8	21	62	394 ♀	60	15.1	0	0.0
9	23[d]	53	1,690 ♀	793	46.9	1	0.05
10	24[d]	64	592 ♀	387	65.3	2	0.3
11	24	84	1,103 ♀	688	62.3	11	0.9
			387 ♂	85	21.9	0	0.0
12	25[d]	31	1,292 ♀	1,182	91.4	11	0.8
13	24	84	799[e] ♂	403	50.4	5	0.6
			188[f] ♀	78	41.4	1	0 5
			340[g] ♀	272	80.0	20	5.8
			205[h] ♀	81	39.5	1	0.5
Totals			10,893	5,946	54.5	139	1.2

[a] Lots 1 to 12 were in the first field trial. Lot No. 13 was in the second field trial.
[b] This lot consisted of 513 birds of which 250 were vaccinated and 263 left unvaccinated for controls.
[c] This vaccine undiluted contained 0.1 gram of tissue per cubic centimeter.
[d] This vaccine undiluted contained 0.25 gram of tissue per cubic centimeter.
[e] White Leghorns and Barred Plymouth Rocks from three to six months old.
[f] White Leghorns and Barred Plymouth Rock pullets four and one-half months old.
[g] White Leghorn pullets from six to seven months old. Egg production was about 60 per cent at time of vaccination.
[h] Barred Plymouth Rock pullets from six to seven months old. Egg production was about 60 per cent at the time of vaccination.

Discussion of Results.—As shown in table 11, a total of 10,893 pullets and cockerels from three to seven months old were vaccinated. Chicken-pox lesions developed at the point of vaccination on 5,946, or 54.5 per cent, of the birds, and about the head of 139, or 1.2 per cent, of the birds. In no case, however, did either the lesions at the point of vaccination or those about the head become extensive or in any way prove harmful. The lesions healed without treatment in every instance within 7 to 15 days after they were first observed.

Table 11 also shows that there was wide variation in the percentage of fowls in the different lots that developed lesions at vaccination point or about the head. The results obtained in lots 1 to 5 that were vaccinated with vaccine No. 20 indicate that the age of the vaccine was a factor responsible for such variation. This vaccine was administered to pullets when it was 31, 50, 58, 67, and 79 days old; and produced vaccination-point lesions on 100.0 per cent, 99.1 per cent, 73.9 per cent, 44.1 per cent, and 25.9 per cent of the fowls, respectively. The percentage of the fowls that developed lesions about the head was 4.8, 6.8, and 3.5, respectively, from the 31, 50, and 58-day-old vaccine and none from the 67 and 79-day-old vaccine.

Differences between vaccines of the same age with respect to the percentage of fowls upon which vaccination lesions were produced were also noted. For example, 58-day-old vaccine No. 20 caused lesions on 73.9 per cent of the fowls vaccinated, while but 18.9 per cent of the fowls vaccinated with vaccine No. 21 when it was the same age were so affected. Similar, though less marked, variations in the results were obtained with other vaccines administered when they were approximately the same age can be seen in table 11.

Variation with respect to the percentage of fowls with vaccination lesions also occurred in two instances in pullets and cockerels that received the same vaccine at approximately the same time. In the first instance, vaccination lesions developed on 25.9 per cent of the pullets that were vaccinated with 79-day-old vaccine No. 20 and on 6.1 per cent of cockerels vaccinated with the same vaccine when it was only three days older. In the second instance, vaccination lesions were produced on 62.3 per cent of the pullets and 21.9 per cent of the cockerels which received 84-day-old vaccine No. 24. That such marked differences between the percentage of pullets and cockerels that develop lesions following vaccination do not always occur, however, is illustrated by the results obtained with lot 13. In this case, vaccination of nearly an equal number of pullets and cockerels with the same vaccine resulted in vaccination-point lesions on nearly the same number of birds of each sex.

The results obtained with the flocks of 340 Leghorn pullets and 205 Barred Plymouth Rock pullets of lot 13 that were the same age showed that the same vaccine might affect different flocks of the same sex differently. In this case vaccination-point lesions developed on 80 per cent of the Leghorns and 39.5 per cent of the Barred Rocks, and head lesions developed on 5.8 per cent of the Leghorns and 0.5 per cent of the Barred Rocks. These flocks differed as regards breed but they were of the same age and were producing at the same rate.

The results of these field trials indicate that the vaccination of young fowls by subcutaneous injection of vaccine containing virulent chicken-pox virus is unlikely to produce *harmful* chicken-pox infection. Chicken-pox lesions may be produced, however, both at the point of vaccination and about the head. Such lesions are very limited in extent and may be expected to heal without treatment within 7 to 15 days. The number of fowls that will develop lesions at the point of vaccination is variable but in most instances relatively great and may include all of the birds vaccinated. The number that develop lesions about the head, however, may be expected to be relatively very small and in many instances such lesions will be entirely absent. The variation in the number of fowls that develop lesions after vaccination is to a considerable extent dependent upon the age of the vaccine at the time of administration. However, variations will occur in the effects of different lots of vaccine of the same age and in the effects of the same vaccine on different lots of birds of the same or opposite sex. It cannot be stated, therefore, that different lots of vaccine that are prepared in an identical manner, stored for the same length of time under the same conditions, and administered in the same dosage, will produce the same reaction in fowls vaccinated with them. Because of the differences in the behavior of apparently identical vaccines, it is impossible to predict the length of time that a vaccine will retain either its property of producing a visible reaction or its potency as an immunizing agent.

A visible reaction in the form of a lesion at the point of vaccination, while not absolutely essential to the production of immunity, usually indicates that the fowl will become immune and perhaps that the immunity will be more lasting than would be the case if no visible reaction occurred. To a certain extent, therefore, the percentage of birds of a flock that develop vaccination-point lesions may be considered as an index of the immunizing value or potency of the vaccine used.

Immunity to Chicken-pox.—During the winter and spring months after vaccination, no chicken-pox occurred in the flock except that which resulted directly from vaccination. Chicken-pox was exceedingly prevalent throughout the state during this time. Furthermore, as stated previously, on one farm chicken-pox had been continuously present for a year before the beginning of these vaccination field trials and on the other farm the disease had occurred during the five preceding winters. These facts suggest that the freedom of the vaccinated birds from chicken-pox resulted from protection afforded them by the vaccine. However, the lack of unvaccinated controls in the flocks makes it impossible to conclude that such was the case.

Evidence that the 250 birds of lot No. 1 of vaccinated fowls were immunized against chicken-pox during the second month after vaccination was furnished by the occurrence of chicken-pox among the 263 non-vaccinated controls that were in the same pen with those vaccinated (see footnote *b*, table 11). The vaccine was administered on April 30. Thirty-four days later the first case of chicken-pox was observed among the controls. Additional cases appeared during the next forty days until 114, or 43.3 per cent, of the controls became infected. All of the vaccinated birds remained free from the disease.

The prevalence of chicken-pox among the unvaccinated susceptible fowls that were allowed to associate with vaccinated fowls having vaccine-produced chicken-pox lesions was an expected and natural occurrence. However, it serves as a concrete illustration of the inadvisability of vaccinating any fowls on a poultry farm with vaccine containing virulent virus unless all of the fowls that are susceptible to chicken-pox are to be vaccinated.

Some of the vaccinated fowls were secured at irregular intervals after vaccination and tested for immunity. The tests consisted in severely scarifying approximately one square centimeter of the comb surface of each bird and applying a suspension in sterile saline of highly virulent chicken-pox virus to the scarified surface. Since birds for this purpose could be secured only as they were to be marketed, it was impossible to follow any definite scheme in making the tests. A total of 165 birds were tested for immunity at intervals after vaccination varying from 54 to 275 days. The data regarding the birds that were tested and the results obtained are summarized in table 12.

TABLE 12

Results of Immunity Tests of Fowls Vaccinated in the Field Trials of Vaccine Prepared from Lesion Tissue

Vaccine No.	Age of vaccine with which fowls were vaccinated (*days*)	Per cent of fowls that had developed vaccination-point lesions	Fowls that were tested for immunity												
			Days after vaccination when tested	Total number tested	Number immune	Number[a] not immune	Fowls that had developed vaccination-point lesions					Fowls that had not developed vaccination-point lesions			
							Number tested	Number immune	Fowls not immune[a]			Number tested	Number immune	Fowls not immune[a]		
									Number	Number with slight lesions	Number with moderate lesions			Total	Number with slight lesions	Number with moderate lesions
20	31	100.0	98	4	4	0	4	4	0	0	0	....	....	....	....	....
			158	4	4	0	4	4	0	0	0	....	....	....	....	....
			192–207	8	8	0	8	8	0	0	0	....	....	....	....	....
20	50	99.1	189	5	3	2	5	3	2	2	0	....	....	....	....	....
			274	5	1	4	5	1	4	2	2	....	....	....	....	....
25	31	91.4	54	5	5	0	5	5	0	0	0	....	....	....	....	....
			140–149	24	18	6	22	16	6	6	0	2	2	0	0	0
20	58	73.9	266–275	5	2	3	3	1	2	1	1	2	1	1	1	0
24	64	65.3	88	10	9	1	5	5	0	0	0	5	4	1	1	0
			178–182	6	2	4	2	2	0	0	0	4	0	4	4	0
24	84	62.3	67	10	10	0	5	5	0	0	0	5	5	0	0	0
			152–161	5	4	1	1	1	0	0	0	4	3	1	1	0
23	53	46.9	110	10	9	1	5	4	1	0	1	5	5	0	0	0
			196–205	25	3	22	8	0	8	3	5	17	3	14	7	7
20	67	44.1	172	10	9	1	5	4	1	1	0	5	5	0	0	0
			257–266	5	1	4	0	0	0	0	0	5	1	4	4	0
21	58	18.9	150	10	4	6	5	3	2	1	1	5	1	4	3	1
			234	4	0	4	0	0	0	0	0	4	0	4	0	4
		Totals.....		165	100	65	97	68	29	16	13	68	32	36	23	13

[a] The lesions on the fowls that were not immune in no case spread beyond the confines of the scarified area nor were otherwise pronounced. They were classified as slight or moderate.

Slight lesions consisted of the formation of a small amount of dry yellowish scab which at no time had the appearance of an active chicken-pox lesion. They were healed in 12 to 18 days after inoculation.

Moderate lesions began development like an active lesion. Drying occurred within a few days, however, and the lesions were entirely healed in 12 to 25 days after inoculation.

Non-vaccinated control fowls were inoculated with each lot of vaccinated birds. In all cases the controls developed pronounced lesions which were still active when the lesions on the vaccinated birds were entirely healed.

As shown in table 12, of the total of 165 fowls that were tested for immunity, 100, or 60.6 per cent, were immune to the infection and 65, or 39.3 per cent, were to some degree susceptible to the infection. Of the 65 fowls that were not entirely immune, 39, or 60 per cent, developed slight lesions and 26, or 40 per cent, developed moderate lesions. The control fowls which were inoculated with the same virus and at the same time as the vaccinated birds developed pronounced lesions that spread to cover an area from three to four times as large as that scarified and that were still active when the inoculation wounds or lesions on the combs of the vaccinated birds were entirely healed. It may be said, therefore, that all of the 165 vaccinated birds that were tested for immunity in from 54 to 275 days after vaccination were either immune or highly resistant to the artificial infection with chicken-pox virus.

The data in table 12 show that 97 of the 165 birds that were tested for immunity were from those that had developed lesions at the point of injection of the vaccine and that 68 were from those that had not developed vaccination-point lesions. Of the 97 birds that had developed vaccination lesions, 68, or 70.1 per cent, were immune; and of the 68 birds that had not developed vaccination-point lesions, 32, or 47 per cent, were immune.

To further demonstrate that the percentage of completely immune fowls was greater among those that had developed vaccination-point lesions than among those that had not developed vaccination-point lesions, the data are grouped in table 13 according to the percentage of fowls which developed vaccination-point lesions. But little difference with respect to the percentage of fowls that were completely immunized was found to exist between the lots of fowls of which 62.3, 73.9, 91.4, 99.1, or 100 per cent developed vaccination-point lesions and those of which 18.9, 25.9, 44.1, or 46.9 per cent developed vaccination-point lesions. Therefore, the fowls are grouped as those of which from 62.3 to 100 per cent (group 1) developed vaccination-point lesions and as those of which from 18.9 to 46.9 per cent (group 2) developed vaccination-point lesions. The data in table 13 are also arranged to show the variation in the percentage of fowls that were completely immunized with respect to the time after vaccination when the test for immunity is made. The number of birds is too small to permit consideration of each lot of birds with respect to the time between vaccination and the test for immunity; therefore, in table 13, grouping is made of those fowls which were tested for immunity within 207 days after vaccination and of those fowls which were tested in from 234 to 275 days after vaccination.

TABLE 13

THE RELATION OF THE PERCENTAGE OF FOWLS THAT DEVELOPED VACCINATION-POINT LESIONS AND OF THE TIME BETWEEN VACCINATION AND THE TEST FOR IMMUNITY TO THE PERCENTAGE OF FOWLS THAT WERE COMPLETELY IMMUNIZED

Group No.	Per cent of fowls that developed vaccination-point lesions	Time after vaccination when tested for immunity *days*	Number of fowls	Number immune	Per cent immune	Fowls that had developed vaccination-point lesions			Fowls that had not developed vaccination-point lesions		
						Total number	Number immune	Per cent immune	Total number	Number immune	Per cent immune
1	62.3 to 100	54 to 207	81	67	82.7	61	53	86.8	20	14	70.0
		234 to 275	10	3	30.0	8	2	25.0	2	1	50 0
		54 to[a] 275	91	70	76.9	69	55	79.7	22	15	67.2
2	18.9 to 46.9	54 to 207	65	29	44.6	28	13	46.4	37	16	43.2
		234 to 275	9	1	11.1	0	0	0	9	1	11.1
		54 to[b] 275	74	30	40.5	28	13	46.4	46	17	36 9

[a] Includes all fowls of group 1. [b] Includes all fowls of group 2.

In table 13, it is again shown that the percentage of fowls that were completely immunized to chicken-pox was greater among those which had developed vaccination-point lesions than among those which had not. The percentage of the fowls developing vaccination-point lesions that were immunized was greater in the group in which a majority developed such lesions than in the group in which a minority developed them. Table 13 also shows that the percentage of fowls that were completely immunized was greater in the former group than in the latter group, even among the fowls that did not develop vaccination-point lesions. These results indicate that the percentage of fowls that become completely immunized from vaccination among those that either do or do not develop vaccination-point lesions will be greater when 60 per cent or more of the vaccinated fowls develop vaccination-point lesions than when less than 50 per cent of the vaccinated fowls develop vaccination-point lesions. This applies particularly to the results with the fowls that were tested for immunity within 207 days after vaccination. These comprised 146 of the 165 fowls, leaving but 19 fowls that were tested for immunity in from 234 to 275 days after vaccination. The percentage of these latter that developed vaccination-point lesions and the percentage that was found to be completely immunized were much less than was the case among the fowls that were tested earlier. The number of birds, however, is

too small to permit accurate comparisons of results obtained with those obtained with birds among which a larger percentage developed vaccination-point lesions or which were tested for immunity sooner after vaccination.

In this discussion of the results of tests for the immunity of vaccinated birds to chicken-pox, particular attention has to be paid to the number of birds that were completely immunized. It should be remembered, however, that all of the fowls which were not completely immunized had a high degree of resistance to the infection irrespective of whether they had or had not developed vaccination-point lesions and of the time after vaccination when the tests for immunity were made. In the majority of cases, the degree of resistance amounted to nearly complete protection against the artificial infection with chicken-pox virus.

CONCLUSIONS

This paper presents the results of studies in the immunization of fowls against chicken-pox by the subcutaneous injection of vaccine prepared by mixing finely ground fresh tissue obtained from cockerels with pronounced comb infection of chicken-pox with a suitable liquid diluent. In these studies approximately one thousand birds that were kept under laboratory conditions and fourteen thousand birds on poultry farms have been utilized.

In the preparation of vaccine, the entire comb, or the lesion and sub-lesion epithelial tissue, either alone or with the addition of the liver, spleen, kidneys, and blood, was used. The lesion and sub-lesion epithelial tissue, alone, however, appeared to be the more satisfactory. As a diluent or vehicle and preservative for the tissue, either 0.5-per-cent phenolized physiologic salt solution or a mixture of equal parts of glycerine and 1.0-per-cent phenolized physiologic salt solution was used. The latter seemed to be preferable. The conclusions which follow are based upon the results of experiments with vaccines prepared from lesions and sub-lesion epithelial tissue and a mixture of equal parts of glycerine and 1.0-per-cent phenolized physiologic saline.

Such vaccine is capable of producing in fowls within 28 days after administration either complete immunity or a high degree of resistance to artificial infection with chicken-pox virus. The immunity or resistance has been shown to last for at least as long as 275 days. The data, however, are insufficient to permit conclusions regarding the percentage of vaccinated fowls that will remain completely immunized for such a long period after vaccination.

The immunizing value of vaccine has been shown to depend upon and to vary according to the amount and virulence of the virus it contains. No method has yet been devised, however, by means of which less than marked differences between the virus content of vaccines can be detected.

The results of the experiments have demonstrated that attenuation of the virus is not necessary to make the vaccine safe for use. However, slight attenuation does not injure the immunizing value of vaccine and may make more remote the possibility of harmful chicken-pox lesions resulting from subcutaneous injection. This may be accomplished by aging vaccine for a short period. Aging also serves to destroy contaminating bacteria. The length of time that vaccine may be aged without the virus becoming too much attenuated will vary according to the concentration of tissue, although variation in this respect also occurs in different vaccines with the same concentration of tissue. It is desirable, therefore, to use no more diluent in the preparation of vaccine than is necessary to facilitate the grinding process. In these experiments, sufficient diluent to make the concentration of tissue in vaccine 0.1 or 0.25 gram per cubic centimeter was found to be satisfactory. The length of time which the virus in vaccine of such concentration withstood aging without too great attenuation varied from 50 to 140 days. In vaccines containing 0.01 gram or less of tissue in a cubic centimeter, however, the virus was entirely destroyed in less than 40 days.

The amount of tissue in an immunizing dose of vaccine may vary within wide limits. The amount that was used most extensively in these experiments and which gave satisfactory results was 0.002 gram. To provide 0.002 gram of tissue in a dose of vaccine, the more concentrated preparation is diluted so that the stipulated amount is contained in one cubic centimeter. The dilution should be made just before administering. For the production of immunity to artificial infection in 28 days after vaccination, one dose of vaccine is as effective as two doses. No data has been obtained, however, regarding the comparative length of the immunity produced by one dose and that produced by two doses.

The subcutaneous injection of vaccine containing an abundance of virulent virus is usually followed by the development of a chicken-pox lesion at the point of injection. Such lesions do not spread to other parts of the body nor otherwise become harmful. When vaccine that is not more than 30 days old is used, vaccination-point lesions will be produced on nearly all of the fowls. When older vaccine is

used, the percentage of fowls which will develop vaccination-point lesions decreases as the age of the vaccine increases. Variations in respect to the percentage of fowls that develop such lesions, however, will occur between different vaccines of the same age and between different lots of fowls that are vaccinated with the same vaccine. Lesions of slight magnitude may develop also about the head of a small percentage of fowls that are vaccinated with vaccine that is not more than 30 days old but they are not apt to occur when older vaccine is used. The occurence of such lesions, however, is subject to variation under the same circumstances as vaccination-point lesions. The lesions may be expected to heal without treatment within from 7 to 15 days without harming the fowl. The largest number of fowls in a flock on which such lesions have occurred after vaccination is 6.8 per cent of 726 fowls.

The percentage of fowls that develop vaccination-point lesions is to some extent an index of the virulence or of the degree of attenuation of the virus in the vaccine. The development of lesions at the point of injection of vaccine is not essential to production of immunity. Fowls which develop these lesions, however, will usually become immunized. All fowls that receive vaccine in which the presence of virulent virus can be demonstrated become either immune or highly resistant to artificial infection with chicken-pox virus. The percentage of fowls that become completely immunized, however, will be greater among those that have developed vaccination-point lesions than among those that have not. Furthermore, the percentage of all fowls that become completely immunized after vaccination, irrespective of whether they do or do not develop vaccination-point lesions, will be greater in flocks in which 60 per cent or more of the fowls develop vaccination-point lesions than in flocks in which a smaller percentage of the fowls develop such lesions. The development of these lesions therefore may be regarded as a favorable vaccination reaction.

The vaccine may be administered to fowls in flocks in which an outbreak of chicken-pox exists without increasing the severity of the lesions in fowls already infected or hastening the spread of the infection among the healthy fowls. Vaccination of such flocks should result in control of the outbreak within 28 days.

Flocks of healthy young fowls from four to seven months old can be vaccinated for the purpose of immunizing them against subsequent infection without danger of inducing harmful chicken-pox infection among them. In such cases, however, all of the susceptible fowls on

the premises must be vaccinated. It would be unwise to use vaccine on a poultry farm on which chicken-pox had never existed unless it was so situated that chicken-pox virus was likely to be introduced on it at any time.

Although the data obtained in these experiments are not sufficient to prove definitely that when young fowls are vaccinated during the summer and fall they will be protected against chicken-pox infection during the following winter and spring, they nevertheless suggest that such may be the case.

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